EDITOR'S VIEWPOINT

Anderson (p. 664) refined his technic for studying the masticatory forces by modifying the calibration. His method utilizes a strain gauge inserted into an inlay. By using conditions simulating those present intraorally, he found it possible to compute the total loads on single teeth.

Anderson (p. 671), using strain gauges in the lower molar teeth on 4 subjects, has measured stress in mastication. While the number of subjects and recordings throw some question on the validity of final conclusions, variation in chewing habits of different individuals is indicated. This technic, and further refinements in it, may give valuable information for more effective dental restoration.

Deuschle and Warkany (p. 674) studied, cephalometrically, heads of rats in which malformations had been induced by severe maternal riboflavin deficiency. There were variations in the degree of retardation of growth in different regions of the jaws, suggesting a factor which may contribute to the production of malocclusion. As the authors carefully point out, congenital malformations can be induced in animals, experimentally, by any of a variety of factors, and in man it is very likely that comparable anomalies are likewise "attributable to a variety of factors rather than to a single cause." This contribution should be of great value to students of teratology, dentofacial anomalies, and orthodontics.

Kreshover and Hancock (p. 685) reinvestigated the abnormal enamel formation in rabbits following inoculation with vaccinia. As in dental abnormalities following parathyroidectomy, hypophysectomy, and nutritional deficiency, a retarded eruption rate seems to be the principal mechanism involved. The similar results seen in the continuously erupting teeth of animals treated by some operative procedures may have a similar cause.

Clark (p. 693) exposed mice to the dust (aluminum oxide) from dental procedures under the airbrasive technic, and to dust from cavity preparation with rotating stones. The 5 mice examined after airbrasive dust exposure showed parenchymatous lesions of pneumoconiosis. The particles were not seen in section and it is assumed that they may have been eliminated by ciliary action with inflammatory reaction occurring distal to them. This problem requires more thorough investigation.

Costich (p. 696) gave mandibular block anesthetic injections to 50 medical students using procaine with 1:50,000 epinephrine and procaine alone. The study confirmed certain clinical impressions. With epinephrine in the solution there is a postinjection drop in diastolic blood pressure. Where the anesthesia is of longer duration, there are more untoward reactions, and a more desirable depth of anesthesia. The significantly shorter duration and lighter anesthesia with procaine free of epinephrine makes its use in routine dental operations undesirable.

Bur design generally has been left to the manufacturer whose desire to produce effective and superior products has been controlled, in part, by economic factors. The dentist and patient, as consumers, have an important influence in this regard. Recently, with the advent of higher speeds of rotation, dental investigators in the field of materials have been stimulated to investigations in this area. Henry (p. 704) studied several factors, i.e., finish, heat treatment and flutes, bur diameter, depth of cutting, and number of blades. The major problem appears to be producing equally sharp edges consistently. With dental investigators in schools, in the National Bureau of Standards, and other institutions cooperating with dental manufacturers, the practitioner can hope for better cutting instruments.

Johnson and Bevelander (p. 714) examined pulp calcifications by histochemical methods and observed that each begins as an organic nidus of reticular tissue and ground substance which becomes calcified. While

acid polysaccharide and a mucoprotein were observed, phosphatase did not appear involved in pulp calcifications. "True" pulp stones (those with odontoblastic processes) were not identified. Diffuse calcifications were limited to the pulp canal. Whether or not calcifications should be considered evidence of retrogressive, or of pathosis, or as protective in character was not defined.

Vargas (p. 723) found statistical differences between the accommodation (the increment of threshold of a tissue stimulated with electric currents of increasing time-constant) of nerves of normal and abnormal pulps. The technic, as other pulp testing methods, should be evaluated against clinical symptoms and histologic changes. As presently conceived, pulp tests are essential, but unreliable, aids to diagnosis. If better methods of pulp testing are demonstrated, the clinician and ultimately the patient will benefit.

Davies (p. 734) examined 497 Pukapukans and found some degree of periodontal disease in 45 per cent of the subjects. This epidemiologic study of periodontal disease again brings to the fore the lack of a generally accepted index for periodontal disturbances. The criteria used appear worthy of further evaluation. The relationship of calculus to gingivitis is to be expected; the association between high erythrocyte sedimentation rates and high serum protein levels and severe periodontal disease are not surprising, but the lower leukocyte counts associated with periodontal disease are not readily explained. While such a study on a small insular population may appear of little significance, at first consideration, this report is worthy of careful review by all with investigational and clinical interest in periodontal diseases.

Asgar (p. 742) presents the chemical analysis of 20 teeth (10 of Greek origin and 10 from local sources). Little has been written in this field since the 1930's when the Rochester group (Hodge, LeFevre, Bale, et al.), Armstrong and Brekhus and Bowes and Murray were actively working on inorganic analyses of teeth. Newer methods should afford more accurate analyses than then available. Asgar found differences in the inorganic composition of Greek and "domestic" teeth. Since the calcium and magnesium were consistently higher and the phosphorus lower in domestic teeth, this variable should be investigated further with consideration to the age, caries susceptibility, and other factors. Perhaps more accurate analyses may show up differences hidden to earlier investigators.

The use of tin (as stannous fluoride) in a dentifrice makes the possible incorporation of tin into enamel more than a curiosity. Brudevold and Steadman (p. 749) found the amount of tin in enamel of unerupted teeth infinitesimal and identified little more in erupted teeth. Some additional tin was found in enamel of teeth bearing amalgam restorations, believed to result from diffusion. The relatively small amounts of tin incorporated into enamel support the hypothesis that if the tin in stannous fluoride does help reduce caries activity it does so as a surface precipitate.

An investigation of the chloride level of salivas of 251 children by Anders (p. 753) showed statistically higher levels associated with higher caries activity. It is suggested that the chloride may act on salivary amylase to produce this relationship. This subject should be investigated further since this study contraindicates the only other report on the subject made in recent years.

An analysis of the urine of children using a stannous fluoride-containing dentifrice, those using a nonstannous fluoride dentifrice, and those consuming communal water supplies at a level of 0.85 p.p.m. showed that the stannous fluoride in the dentifrice did not increase the urinary fluoride output and that the urinary fluoride output and the urinary fluoride level of these children was 75 per cent less than those consuming water with 0.85 p.p.m. of fluorine. Schweinsberger and Muhler (p. 760) consider these data as evidence that the routine use of stannous fluoride dentifrice is not associated with any "real increase" in skeletal fluorine storage.

Wantland (p. 763) observed a marked decrease in the numbers of parasites in hamsters receiving 2 to 10,000 p.p.m. of sodium fluoride. In vitro, living Protozoa and Rotifera were then subjected to various concentrations of sodium fluoride. These organisms survived and reproduced in 2 to 1,000 p.p.m. of sodium fluoride but at 1,700 to 10,000 p.p.m. the organisms died. The Rotifera reacted in such a manner that their use in

a biologic test is proposed to differentiate waters with less than 10 p.p.m. of sodium fluoride from those with 20 or more p.p.m.

It has been postulated that enamel treated with fluorides may form a calcium fluoride or a fluorapatite. Fischer, Ring, and Muhler (p. 773) used improved x-ray diffraction methods on treated powdered enamel and were unable to identify fluorapatite. The investigation did not extend to enamel of subjects receiving fluorides, systemically, during tooth development. As these technics are refined more information may be gained, but it should be pointed out that apparatus and technics, alone, will not solve the problem. The methods must be applied by investigators thoroughly familiar with the technics and interpreted by qualified persons. The caution indicated by the authors who developed the instruments should be reflected in studies by others.

A series of 3 papers on the formation of lactic acid in dental plaques is a joint effort of Northwestern University, the Great Lakes Naval Training Center's Research Facility, and the University of Witwatersrand. Moore, Carter, Dunn, and Fosdick (p. 778) developed a method for in situ measurement of pH and lactate content of the plaque, and found that increase in lactate content following use of a sucrose mouth wash paralleled pH fall. Nearly all of the pH decrease could be accounted for by lactate increase. Clement, Plotkin, and Fosdick (p. 786) used the technic on African Bushmen. These interesting and extremely primitive people practice no agriculture or herding, living "on the land" and hunting with primitive bows and arrows. They, therefore, eat game, melons, berries, roots, grubs, and ants, when they can find them, starving at other times. The Bushman is caries inactive, has poor oral hygiene (in line with his general hygiene,) much calculus, and rampant periodontal disease. His oral flora is not acidogenic and his dental plaques and saliva have much different buffering capacity than saliva of caries-active individuals. One might ask if both acidogens and sugar would need to be introduced to induce caries in these people as in the germ-free animals. Again, using the in situ plaque test, Englander, Carter, and Fosdick (p. 792) found a correlation between pH, normal lactate, and free lactate in caries-active and caries-immune young American subjects. They have proposed that plaque pH values of 5.7, or above, and 3 mM per Kg. of free lactic acid are harmless. Such studies on immunes offer great promise in the field of caries research. The paper of Green and Dodd (*J. D. Res.* **35:** 572, 1956) may be correlated with this.

Charlton and Spies (p. 800) compared tomato peptone agar and Rogosa S. L. medium for their selectivity for lactobacilli from saliva. They found the synthetic medium (Rogosa's) more selective than tomato-peptone agar. They consider it suitable for routine lactobacillus counts. Although this study compared two media for salivary lactobacillus counts, it did not evaluate either of the types of counts for their efficiency in predicting dental caries. Greater value will be derived from evaluating technics and materials against the progress of caries in patients. Charlton and Spies' results should be compared with those of Snyder, Suher, Porter, Claycomb, and Gardner (*J. D. Res.* **35:** 332, 1956).

Mortell and Peyton (p. 804) examined sections of teeth for Hunter-Shreger bands by the replica method. They confirm many of the opinions gained from conventional microscopic studies. The concentric areas observed in cuspal areas were revealed by replica technic, but the bands were sometimes observed where there was no change in enamel rod direction. The replica studies suggest difference in mineralization of the alternating bands, not optical phenomena alone.

The Executive Proceedings of the International Association for Dental Research are published in this issue. We have departed from the custom of publishing the abstracts of the papers presented at the Annual Meeting for two reasons. First, the abstracts were published prior to the meeting and are available to all who are interested from the Secretary of the Association. Secondly, there is a huge backlog of complete, original articles awaiting publication. It is our belief that since the abstracts are available they should not displace previously completed research papers. We hope that the abstracts can be reproduced by a relatively inexpensive method and made available in the same page size as the JOURNAL for those who wish to bind them with the volume.

H. B. G. R.

MEASUREMENT OF STRESS IN MASTICATION. I

D. J. ANDERSON, PH.D., M.SC., B.D.S.

Physiological Laboratory, Guy's Hospital Medical School, London, S. E. 1, England

IN a previous paper[1] a method was described by which masticatory loads could be recorded, using a resistance wire strain gauge small enough to be accommodated in a molar tooth. Since then, this apparatus has been constructed for 7 subjects, and data from 4 of them are presented in this paper.

METHOD

In every subject, all of whom had complete or nearly complete dentitions, a lower molar tooth was chosen which had already been fairly extensively restored. Two lower left first molars, 1 lower left second molar and 1 lower right first molar were used. The preparation of the chosen tooth and the construction of the strain gauge apparatus followed the technic previously described. Records were obtained with a Wheatstone bridge circuit, D.C. amplifier, and oscilloscope and were photographed. Calibration of the strain gauge unit took place outside the mouth before and after every chewing sequence, and, if the calibrations differed, the records obtained in the mouth were discarded.

Three materials were chosen for the investigation: biscuit of a fairly hard texture, raw carrot, and cooked meat. The biscuits, approximately 2 inches in diameter and 3/8 inch thick, were halved and then chewed without preliminary incisor biting. The carrot and meat were cut into 1/2 inch cubes and were not subjected to incisor biting before chewing. It was found necessary, in view of the disturbing effect of temperature change on the stability of the strain gauge, to warm the carrot and meat before chewing by placing them for a few minutes in water at 37° C.

The subject was provided with a switch to signal the beginning and end of the chewing sequence, the signal being recorded by a small elevation of the oscilloscope time tracing for the duration of the chewing sequence. Twenty pieces of each material were chewed, usually spread over several sessions.

RESULTS

Qualitative.—Examples of the oscilloscope records are shown in Fig. 1. It will be seen that the recorded loads tend in many cases to be larger toward the end of the sequence than at the beginning and in some there is a wave form superimposed on this trend. In addition, the last 1 or 2 loads are frequently larger, more prolonged, or separated from the rest by a longer interval than

This paper is based on material in a thesis presented by the author for the degree of Doctor of Philosophy in the University of London.

Received for publication April 11, 1955.

usual. An attempt was made to bring out these trends more clearly in the following way: The individual loads were measured by reference to the calibration curves and their position in time determined from the time tracing on the photographic record. The duration of individual chewing sequences varied by several seconds around a mean of approximately 10 seconds, and,

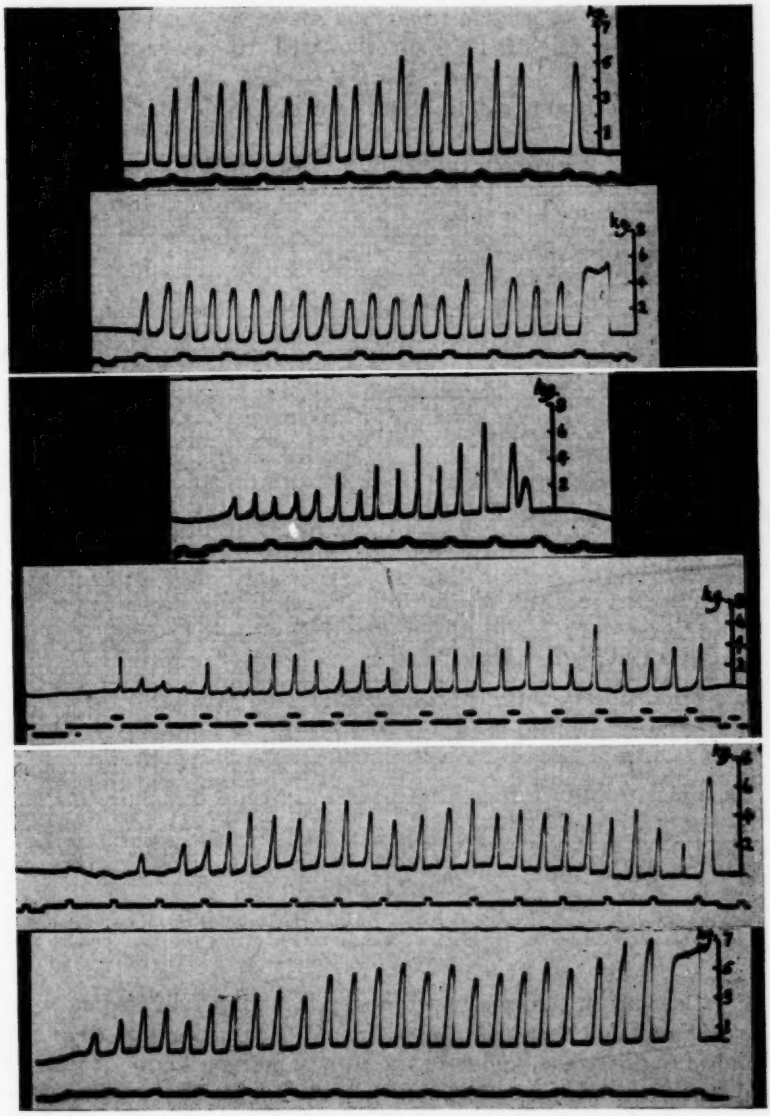


Fig. 1.—Examples of oscilloscope records from the 4 subjects chewing biscuit, carrot, and meat. The upper tracing is the strain gauge response with a calibration scale included. The lower tracing is elevated at one-second intervals and is raised bodily for the duration of the chewing sequence.

to avoid the effect that such variation might have in obscuring trends shown in individual sequences when all the loads were plotted together, the duration of every chewing sequence was scaled to 10 time units. Scatter diagrams of the loads obtained from 20 chewing sequences were then plotted using the new time scale. Fig. 2 shows how such treatment of the biscuit results obtained in Subject 1 brings out the tendency for the recorded loads to rise toward the end of the sequence. A similar trend was shown by 3 out of the 4 subjects with biscuit, but it was hardly noticeable with the other materials. With carrot, Subject 1 showed a trend toward smaller loads at the end of the sequence, but this tendency was not seen in the other subjects.

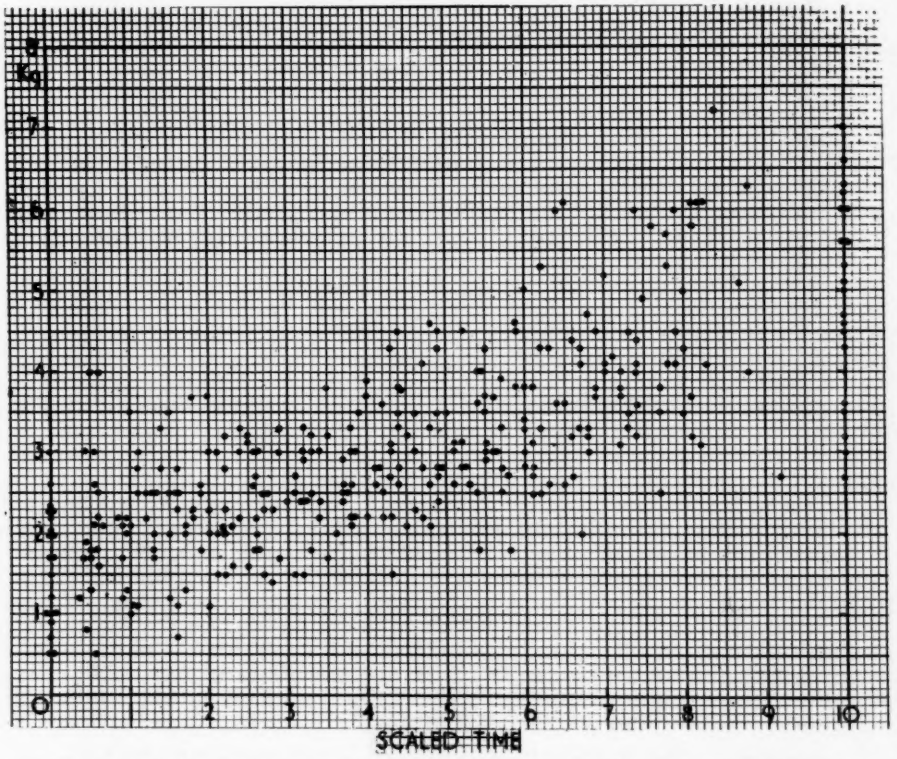


Fig. 2.—Scatter diagram of all the loads applied by Subject 1 in the twenty chewing sequences with biscuit. Loads in kilograms. The duration of all the sequences scaled to 10 time units.

Observation of the subjects in whom the last thrust differed in some way from the rest revealed that this thrust coincided with the movement of swallowing and must therefore be due to the firm apposition of the teeth in fixing the mandible while the floor of the mouth is elevated. The patterns shown by the 4 subjects exhibit individual characteristics, Subject 2, for example, showing a prolonged thrust at the end of every sequence whatever the material being chewed.

Quantitative.—The method provides no direct evidence as to the total loads taken by the chosen tooth, and the area of the strain gauge platform is not the same, nor even the same fraction of the total load-bearing area, in every subject. The results are therefore not comparable quantitatively as they stand.

Since the area of the strain gauge platform can be measured, it is possible, with the results obtained so far, to calculate the pressure per square millimeter of the platform. It is reasonable to assume that the distribution of load is uniform over the load-bearing surface of the tooth with most food materials, and so these calculations provide a measure of the pressure to which the tooth is subjected. Such results may be legitimately compared in the four subjects.

The Maximum Pressure Per Chewing Sequence.—The maximum thrust recorded in every chewing sequence has been converted to a figure for the pressure per square millimeter, and a mean value calculated for the 20 sequences with every food. The results are given in Table I, and they suggest that the values for meat are lower than for the other materials. However, testing the meat values against the closer of the other 2 shows a statistically significant difference at a probability level of 1 in 100 in Subjects 1 and 4 only.

TABLE I

MEAN MAXIMUM PRESSURES PER SQUARE MILLIMETER OF GAUGE UNIT PLATFORM FROM TWENTY SEQUENCES

(Pressures in kg./sq. mm. σ = standard deviation)

SUBJECT	PRESSURE	σ
	Biscuit	
1	0.81	± 0.17
2	1.66	± 0.18
3	1.18	± 0.26
4	0.67	± 0.14
	Carrot	
1	0.76	± 0.10
2	1.75	± 0.44
3	1.21	± 0.19
4	0.66	± 0.14
	Meat	
1	0.40	± 0.07
2	1.61	± 0.31
3	1.01	± 0.39
4	0.49	± 0.20

Total Pressure Per Sequence.—The total pressure per sequence is calculated by adding up the pressures calculated from the recorded biting thrusts. The mean values are given in Table II. As might be expected from Table I, the values obtained from Subject 2 are in every case higher than from the other 3 subjects, in whom little variation is shown.

Day-to-Day Variation.—In an attempt to assess the constancy of behavior in chewing, the biscuit results provide useful data, since the biscuits used were all from the same batch and had been kept in an airtight container. The extremes of day-to-day variation in the mean total pressures are shown in

Table II. In 3 out of 4 subjects with biscuit these values are significantly different at a probability level of 1 in 100. Table III shows the mean number of recorded thrusts and the extremes of day-to-day variation. The means obtained with biscuit in the 4 subjects show much less scatter than those with carrot and meat. However, although the day-to-day means do not differ significantly in 3 out of the 4 subjects with biscuit, the same is true in 2 out of 3 subjects with carrot and in 2 out of 3 subjects with meat.

TABLE II

MEAN TOTAL PRESSURE PER SQUARE MILLIMETER OF GAUGE-UNIT PLATFORM PER CHEWING SEQUENCE

(Pressures in Kg./sq. mm. σ = standard deviation. The extremes of day-to-day variation are shown. The values for Student's "t" and probability [P] are given)

SUBJECT	PRESSURE	σ	EXTREMES OF DAY-TO-DAY VARIATION		"t"	P
			DAY 1	DAY 2		
			Biscuit			
1	7.7	±1.77	6.07	11.25	Obviously different	
2	20.8	±2.95	19.86	23.70	1.12	0.3-0.2
3	6.3	±1.41	4.48	7.08	3.67	0.01-0.001
4	5.5	±1.80	7.56	4.29	6.99	Less than 0.001
			Carrot			
1	8.4	±1.18	All on same day			
2	23.4	±11.62	12.6	37.6	Obviously different	
3	7.8	±1.90	9.7	6.37	2.79	0.05-0.02
4	8.4	±1.99	10.1	6.86	5.38	Less than 0.001
			Meat			
1	3.7	±1.17	All on same day			
2	19.5	±2.20	16.78	30.7	Obviously different	
3	9.3	±1.55	13.88	7.08	4.7	Less than 0.001
4	8.0	±2.37	4.97	9.51	7.03	Less than 0.001

TABLE III

MEAN NUMBER OF RECORDED CHEWING MOVEMENTS FROM TWENTY SEQUENCES

(The extremes of day-to-day variation are shown with the values for Student's "t" test and probability [P] obtained in examining the day-to-day values. σ = standard deviation)

SUBJECT	NUMBER OF MOVEMENTS	σ	EXTREMES OF DAY-TO-DAY VARIATION		"t"	P
			DAY 1	DAY 2		
			Biscuit			
1	16.20	±1.85	14.87	18.00	2.14	0.1-0.05
2	17.15	±1.50	16.66	18.66	2.14	0.1-0.05
3	17.35	±1.84	18.33	16.00	4.62	Less than 0.001
4	15.10	±1.86	14.25	16.30	2.08	0.1-0.05
			Carrot			
1	16.35	±1.38	All on same day			
2	23.35	±4.90	28.50	19.00	5.01	Less than 0.001
3	13.35	±1.66	12.12	14.00	2.41	0.05-0.02
4	23.75	±2.75	22.88	25.00	1.18	0.3-0.2
			Meat			
1	12.15	±2.21	All on same day			
2	21.95	±3.64	21.25	24.75	1.89	0.1-0.05
3	17.70	±3.69	16.20	19.22	1.75	0.2-0.1
4	35.60	±6.50	30.83	38.88	5.1	Less than 0.001

Comparison Between Carrot and Meat Records.—Since both of these materials were taken in half-inch cubes it is reasonable to expect that any difference which might be established in their treatment would be due to differences in the characteristics of the material. Table IV shows the results of Student's "t" test on the mean total pressures and mean number of chewing movements with carrot and meat. In only one subject (Subject 1) are the pressure values obviously different, while the number of chewing movements are different in 3 out of 4 subjects. However, in 2 of these 3, meat requires more chewing movements, and in the third less chewing movements, than carrot.

TABLE IV

THE MEAN TOTAL PRESSURES AND THE MEAN NUMBER OF RECORDED CHEWING MOVEMENTS. A COMPARISON BETWEEN THE CARROT AND MEAT RESULTS

(Pressure in Kg./sq. mm. σ = standard deviation. Student's "t" and probability [P] values are given)

SUBJECT	CARROT σ	MEAT	"t"	P
		Mean total pressures		
1	8.4 ± 1.18	3.7 ± 1.17	Obviously different	
2	23.4 ± 11.62	19.5 ± 2.20	1.28	0.3-0.2
3	7.8 ± 1.90	9.3 ± 1.55	1.23	0.3-0.2
4	8.4 ± 1.99	8.0 ± 2.37	Obviously *not* different	
		Mean number of chewing movements		
1	16.35 ± 1.38	12.15 ± 2.21	7.2	Less than 0.001
2	23.35 ± 4.90	21.95 ± 3.64	1.02	0.2-0.1
3	13.35 ± 1.66	17.70 ± 3.69	4.8	Less than 0.001
4	23.75 ± 2.75	35.6 ± 6.50	Obviously different	

DISCUSSION

Since the recording device used in this investigation occupies only a small portion of the occlusal surface of 1 tooth, the results must be interpreted with caution. Variations in the recorded loads do not necessarily indicate variations in the masticatory effort as a whole. The rising tendency shown by the recorded loads may indicate that the food is chewed mainly on other teeth than the strain gauge tooth in the early stages of the sequence. Yurkstas and Curby,[2] using strain gauges in 3 teeth of artificial dentures, suggest that some foods such as hard bread are chewed initially in the premolar region, moving backward as they become softer.

Alternatively, since the strain gauge apparatus only records the vertical component of each thrust, the smaller initial loads may be due to lateral dissipation of the force in breaking large pieces of material in the early stages of chewing. A third possibility, that the total effort increases toward the end of the chewing sequence, has not been overlooked, although it seems unlikely.

The mean maximum pressure per sequence and the mean total pressure per sequence are in every case higher in Subject 2 than in the other subjects in whom remarkably little variation is shown. The high pressures recorded for Subject 2 do not necessarily indicate that the total loads taken by the tooth are correspondingly large.

CONCLUSIONS

The results obtained so far indicate that the differences between the chosen materials are not sufficiently great to require obviously different treatments as shown by the maximum pressure per sequence, total pressure, and number of chewing movements.

As might be expected, the patterns recorded show some individuality, but the quantity of data available so far does not reveal how much this individuality depends on the food and how much on the performer. When sufficient data have been amassed to establish more definite patterns, it will then be possible to use the technic for physiological studies in the control of mastication and for clinical investigation.

SUMMARY

Masticatory stresses have been recorded in 4 subjects using a resistance wire strain gauge apparatus accommodated in inlays in lower molars. The stresses have been expressed in kilograms per square millimeter of occlusal surface used for recording.

In 1 of the 4 subjects, the total pressure exerted per chewing sequence is strikingly greater than in the other subjects with all 3 food materials tested.

No consistent pattern of treatment emerges as far as the pressures are concerned, even with biscuit which was from the same batch for the whole investigation. When the numbers of recorded chewing movements are examined, however, the variation from subject to subject is much less marked with biscuit than with the other materials.

I wish to acknowledge the great patience of my subjects in this investigation.

REFERENCES

1. Anderson, D. J.: A Method of Recording Masticatory Loads, *J. D. Res.* **32**: 785, 1953.
2. Yurkstas, A., and Curby, W. A.: Force Analysis of Prosthetic Appliances During Function, *J. Pros. Den.* **3**: 82, 1953.

MEASUREMENT OF STRESS IN MASTICATION. II

D. J. ANDERSON, PH.D., M.SC., B.D.S.

Physiological Laboratory, Guy's Hospital Medical School, London, S. E. 1, England

THE method for recording masticatory forces described in 1953[1] was not considered satisfactory as it stood, since it only provided data for the determination of pressures on a small part of the occlusal contact surface of the chosen tooth. It was thought desirable, both from physiologic and clinical viewpoints, to obtain information concerning the vertical loads applied to a whole tooth rather than a small portion of it. Such a goal could only be attained with certainty by using an entire tooth crown for recording purposes, but this approach was rejected because a method requiring such destruction of tooth substance would be even more limited in its application than the present one and might require the use of pulpless teeth. It was therefore decided to modify the technic already developed so that, assuming the load-bearing area between the teeth during chewing to be approximately the same as the area of contact with the teeth in centric occlusion, the loads on the whole tooth could be determined.

The modification in the technic was limited to the calibration. As already described,[1] the strain gauge unit was calibrated outside the mouth by applying known loads to the central platform of the gauge unit and recording the oscilloscope deflections obtained. In the new technic the strain gauge is calibrated under conditions which approach very closely to those prevailing in the mouth. Two copper-plated amalgam models were prepared, one of the strain gauge tooth, and the other of the occlusal surfaces of the two opposing teeth. The strain gauge unit was inserted into its cavity in the tooth model and covered as usual with an inlay. The two models were trimmed so that their long axes were vertical, and after fixing their occlusal relationship by means of a wax bite, their relative positions were secured by a plaster of Paris splint. Then, with the models inverted for convenience and with the strain gauge in position, as shown in Fig. 1, a calibration curve was constructed. In this method the calibrating loads were not borne entirely by the strain gauge platform, but shared by all the areas of contact between the strain gauge tooth and its opponents.

Only Subjects 1 and 2 of the previous study,[2] were available for the necessary preliminary steps when this modification in calibration technic was devised, and in order to make use of the results already obtained both methods of calibration were used and curves were constructed. In subject 1 the two

This paper is taken from a thesis presented for the degree of Doctor of Philosophy in the University of London.

Received for publication April 11, 1955.

curves differed by a factor of 2.8 and in Subject 2 by 1.9. Accordingly, previously obtained results were multiplied by these factors to obtain whole tooth loads.

RESULTS

Maximum whole-tooth loads: Table I shows the mean maximum whole-tooth loads per sequence calculated in the manner already indicated.

Total tooth loads per sequence: Table II shows the mean values for the total whole-tooth loads obtained from 20 sequences with the 3 food materials.

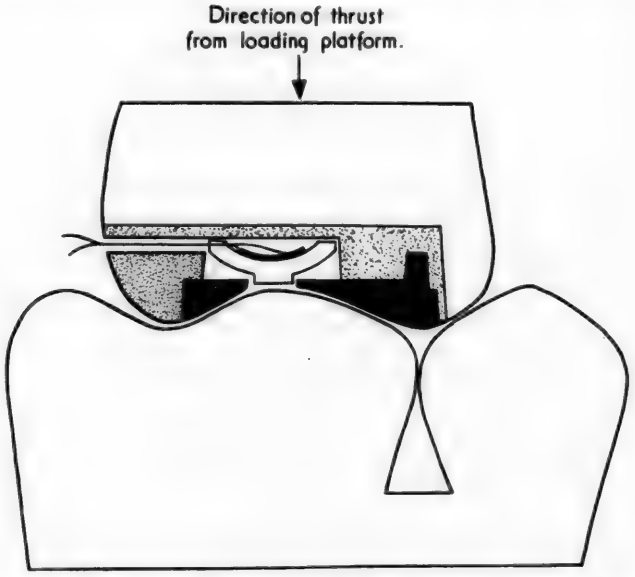


Fig. 1.—Diagram showing the arrangement of the models of opposing teeth for calibration of the strain gauge unit. For convenience the models are inverted. The strain gauge unit is shown in position in the permanent inlay (lightly shaded). The covering inlay is shown darkly shaded.

DISCUSSION

In contrast with the gauge-unit pressures in Subjects 1 and 2 previously reported,[2] the values for whole-tooth loads, both maxima per sequence and total per sequence, show a remarkable similarity. Only the meat values in Subject 1 differ strikingly from the remainder in this subject and from those of Subject 2. It is of interest to compare the figures for maximum masticatory loads with the most recent measurements of maximum biting forces obtained by Howell and Manly[3] using an electronic strain gauge apparatus. According to these workers, the maximum biting force recorded on the first molar was 198 lb. (approximately 90 Kg.). It is not surprising that masticatory loads are smaller since there is no subjective evidence of great effort being employed while chewing ordinary materials. It is interesting to compare the maximum values determined in these experiments with values obtained by Yurkstas and Curby[4] in subjects wearing artificial dentures. These workers recorded maxi-

mum masticatory loads of approximately 12 Kg. on a single tooth although the more common maximum loads were 6 to 8 Kg. They report average loads for most foods of 0.3 to 1.8 Kg. and these values are well below the lowest whole-tooth loads determined in the subjects of this investigation.

TABLE I

MEAN MAXIMUM WHOLE-TOOTH LOADS FROM 20 SEQUENCES IN SUBJECTS 1 AND 2. LOADS IN KILOGRAMS

	SUBJECT 1		SUBJECT 2			
	LOAD	σ	LOAD	σ	"t"	P
Biscuit	14.9	± 3.02	11.4	± 1.24	4.77	less than 0.001
Carrot	13.7	± 1.60	12.0	± 3.01	2.23	0.05-0.02
Meat	7.2	± 1.27	11.6	± 2.58	6.91	less than 0.001

σ = standard deviation.
t = Student's "t."
P = probability.

TABLE II

MEAN TOTAL WHOLE-TOOTH LOADS PER CHEWING SEQUENCE. LOADS IN KILOGRAMS

	SUBJECT 1		SUBJECT 2			
	LOAD	σ	LOAD	σ	"t"	P
Biscuit	138.1	± 31.9	142.2	± 19.8	0.739	0.5-0.4
Carrot	149.4	± 20.3	160.1	± 79.4	0.585	0.6-0.5
Meat	66.5	± 21.0	134.0	± 47.6	5.8	less than 0.001

σ = standard deviation.
t = Student's "t."
P = probability.

CONCLUSIONS

The modification in calibration technic for the resistance strain gauge apparatus which has been described in this report offers a means of measuring the vertical loads taken by a single tooth during mastication. It is claimed that the method allows as close approach to the true values as is possible having regard to the desirability of using technics which require the minimum deviation from normal. The method does not reveal any striking differences in the treatment of the material in terms of maximum and total loads, except in Subject 1 who applied obviously smaller loads to meat than the other 2 materials. It is not possible to draw any general conclusions with the present small series of results.

REFERENCES

1. Anderson, D. J.: A Method of Recording Masticatory Loads, *J. D. Res.* 32: 785, 1953.
2. Anderson, D. J.: Measurement of Stress in Mastication. I, *J. D. Res.* 35: 664, 1956.
3. Howell, A. H., and Manly, R. S.: An Electronic Strain Gauge for Measuring Oral Forces, *J. D. Res.* 27: 705, 1948.
4. Yurkstas, A., and Curby, W. A.: Force Analysis of Prosthetic Appliances During Function, *J. Pros. Dent.* 3: 82, 1953.

CONGENITAL DENTOFACIAL MALFORMATIONS IN RATS INDUCED BY MATERNAL NUTRITIONAL DEFICIENCY

A Cephalometric Appraisal

FREDERICK M. DEUSCHLE AND JOSEF WARKANY

From the Departments of Anatomy and Pediatrics, and the Children's Hospital Research Foundation, College of Medicine, University of Cincinnati, Cincinnati, Ohio

STUDENTS of dentofacial growth have passed from the qualitative to the quantitative phase of study sufficiently to employ some of the tools of the exact sciences. Orthodiagraphic studies, both profile and frontal, have become common methods of relating cranium to face, jaw to jaw, and teeth to jaws in the growing head. Anatomic and craniometric landmarks and planes are in general use and well known. An analysis of the angular relations of these planes is particularly useful in the comparison of deviations from the normal when the absolute measurements of craniofacial structures vary. This method permits comparison of variations within the same species and of variations among different species.

The basic pattern of dentofacial growth is laid down in the genetic constitution of the organism, but even in mammals environmental forces exert their influence upon embryonic development in prenatal life as soon as the zygote is formed. Since the role of the prenatal intrauterine development is not easily observable, "congenital" and "hereditary" characteristics were often thought to be identical! However, it is now well established that the prenatal, intrauterine environment influences embryonic growth and differentiation not merely by gross mechanical forces but also by more subtle chemical, nutritional, toxic, or actinic processes. The organism which presents itself at birth is a product of genetic and prenatal environmental factors. Postnatally the growing structures are further subjected to a host of environmental forces. By the time the teeth erupt the dentofacial relations have become dependent upon so many interacting processes that analysis of anomalies has become difficult. It is often not possible to establish which factors are primary, secondary, or tertiary in the production of dentofacial disorders.

The occurrence of congenital malformations in the jaws of experimental animals offers material for analysis which is free of postnatal influences. In the newborn young described below, a syndrome of skeletal malformations was induced by maternal riboflavin deficiency.[1-3] A definite pattern of anomalies of the jaws and teeth is included in this syndrome.[4] Shortness of the mandible and maxilla, cleft palate, and severe anomalies of the incisor

This study was supported in part by grants from the Nutrition Foundation, Inc., New York, the National Vitamin Foundation, Inc., and the National Institute of Arthritis and Metabolic Diseases (grant number A-427) of the National Institutes of Health, Public Health Service.

Received for publication May 31, 1955.

teeth are observed. The skeletal malformations are determined before chondrification and ossification take place,[5] but the ability of the abnormal fetus to form dental tissues is not lost unless the supporting skeleton is severely affected.[4]

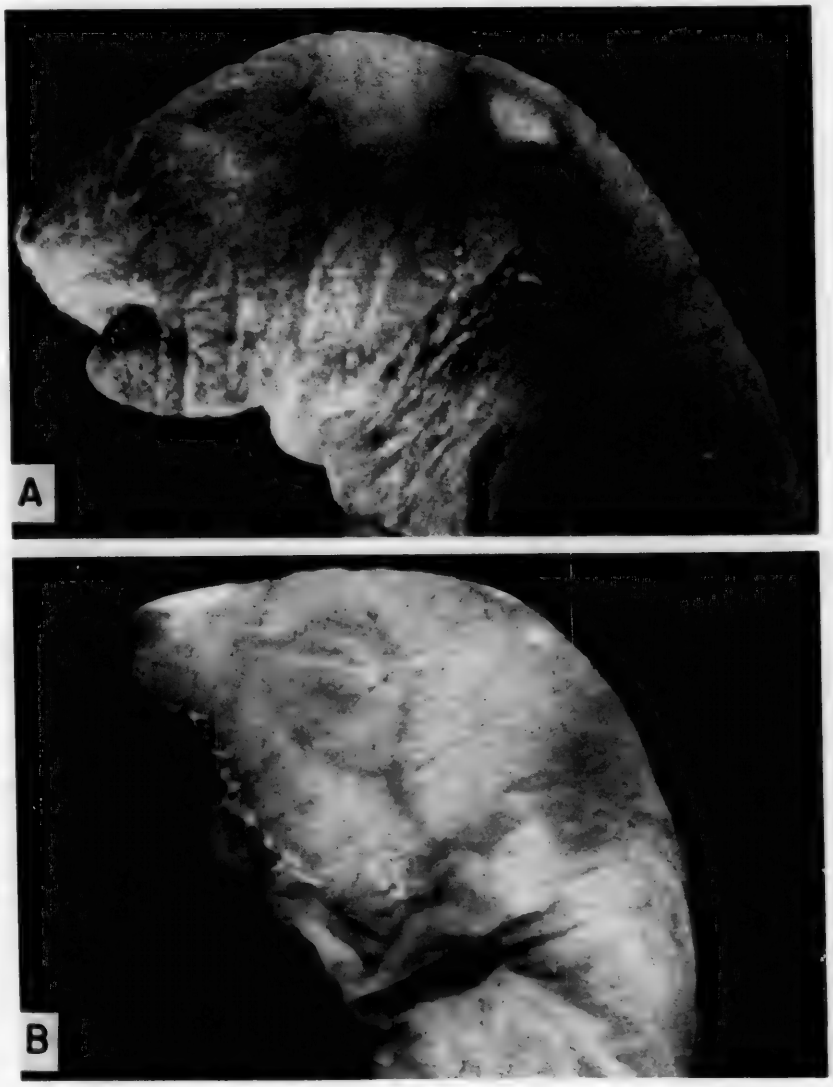


Fig. 1.—Comparison of head configuration of a normal newborn rat (*A*) and a newborn young of a riboflavin-deficient mother (*B*). Note the difference in development of premaxillary and mandibular regions.

The present study was undertaken in order to compare the profile facial pattern of newborn riboflavin-deficient rats with that of normal control neonates. To compare the basic facial patterns, this study employs measurements of

angles and angular relationships instead of estimations of linear dimensions. This method eliminates differences due to absolute size and permits comparison of large and small animals. As a rule, the young of riboflavin-deficient animals are almost as large as the control animals at birth, but there are exceptions to this rule and some interesting abnormal specimens are reduced in size.

METHODS

The maternal diets and the technic of breeding employed for the induction of the anomalies to be described have been published.[1, 3] Under optimal conditions about one-third of the offspring of riboflavin-deficient mothers show congenital skeletal malformations. It was found that of 100 abnormal young, 80 had various degrees of shortening of the mandible (Fig. 1, *B*) and 44 posterior cleft palate.[2] Since the pattern of anomalies was rather homogeneous, a few representative animals could be selected for analysis. The animals used were prepared in two ways. One group was cleared[6] so that the osseous skeleton became clearly outlined. Another group was serially sectioned. Photographs of the cleared heads oriented in profile amount to orthodiagraphic projections which make possible examination of certain angular relations ordinarily obscured in the neonate by the soft tissues. Roentgenographic profile studies in the uncleared specimens proved unsatisfactory since the difference of densities was not sufficient. We are cognizant of artificial changes created by the process of clearing in the anteroposterior relationship of the mandible to the maxilla. In Fig. 2, *A* a slight mandibular protrusion is observable in the control specimen, a condition which does not actually exist in the normal neonate (Fig. 4, *A*). Since, however, all animals of this group were subjected to identical processing, the artificial protrusion exists equally in all the specimens examined and it was felt that the results of the observations were valid. To test this assumption histologic sagittal sections of the animal were included in the study to allow a profile analysis of all the structures near the median plane (Fig. 4). No consideration was given in this study to molding of the head by the birth process. However, it must be pointed out that the young were delivered by the mothers and not obtained by cesarean section. The specimens selected were in a good state of preservation and showed, after clearing, smooth contours and no obvious distortions.

RESULTS

Fig. 2, *A* shows the photograph of a cleared newborn control animal oriented in profile. The normal development of the upper jaw and of the well-developed mandible can be seen. Attention should be directed to the development of the nasal bone and the premaxilla as seen in the normal cleared specimen. The unerupted upper and lower incisors and the molars are recognizable. The length and the height of the mandibular corpus should be noted. In Fig. 2, *B* a mildly abnormal animal is illustrated. There is only a slight shortening of the upper jaw but a marked reduction of the mandibular corpus. The chief loss affects the interdental diestema which

Fig. 2.—Heads of cleared specimens of newborn rats. *A*, Control; *B* to *D*, varying degrees of abnormality.

appears reduced in length and in height. The loss of height can be attributed to failure of development of the upper border while the lower border of the corpus is continuous with that of the ramus as in the normal. The ramus with its coronoid, condyloid, and angular processes shows no aberrant development. The bones of the orbit and of the calvarium appear normal. Fig. 2, *C* represents an intermediate degree of abnormality. Little gross change is observable in the maxillary region over the preceding animal (Fig. 2, *B*), but the mandible is further reduced. Its antemolar region shows a decrease in height by more than one half, if compared with the control. No incisor tooth is visible at the anterior extremity of the mandible. The molar region is relatively well developed and the ramus and its processes appear normal. Fig. 2, *D* shows the extreme degree of deformation in this series. The maxillary region is further shortened and the mandibular corpus anterior to the molar region reduced to a rudiment. There is also a reduction in mass in the molar area, but the ramus is little affected.[4]

Upon the photographs of the cleared animals maxillofacial triangles[7] were traced (Fig. 3), using the following 3 sides: (1) Bolton plane[8] as the cranial base line (BN); (2) facial line (NM) from nasion to the inferior alveolar point (IA); and (3) the mandibular line (MB), a tangent to the inferior border of the mandible continued anteriorly to meet the facial line at M and posteriorly to the cranial base line at B. Three additional lines were drawn from the nasion, one to the superior alveolar point or prothion (P), another to the anterior inferior molar point (A), and a third to the internal angle point of the mandible (I). All the angles at N and the angle at B were measured in the control and abnormal animals.

In the control animal (Fig. 3, *A*) angle MNB is 111 degrees. The corresponding angle in the abnormal animal *B* is 83 degrees (Fig. 3, *B*), in the abnormal animal *C*, 78 degrees (Fig. 3, *C*) and in the most abnormal animal *D* only 65 degrees (Fig. 3, *D*), the maximal loss in the series of abnormal animals being 46 degrees. The angle INB is in the control 43 degrees and in the abnormal animals 42 to 40 degrees, which is no significant deviation from the normal. Therefore, the reduction of angle MNB in the abnormal animals is due to reduction of angle MNI or of its component angles MNA and ANI.

In the abnormal animal *B* angle MNI is reduced from 68 degrees of the normal to 41 degrees, in *C* to 38, and in *D* to 25 degrees. Angle MNA which is in the normal 38 degrees, is reduced in *B* to 23, in *C* to 20, and in *D* to 10 degrees. Angle ANI which is in the normal 30 degrees, is only 18 degrees in *B*, 18 degrees in *C*, and 15 degrees in *D*. Expressed in percentage, the loss of angle MNI of animal *D* amounts to 64 per cent. However, this loss is not evenly distributed between the anterior and posterior parts of the corpus. The loss of angle MNA is 73 per cent, and the loss of angle ANI only 50 per cent. It is observed that angle NBM is 33 degrees in all animals.

The other facial angle PNB is in the control 114 degrees; in the abnormal animal *B*, 110 degrees; in animal *C*, 108, and in the extremely deformed

animal *D*, 100 degrees. While maximal loss of angle MNB amounted to 41 per cent, that of angle PNB is only 12 per cent.

In Fig. 4 sagittal sections through the heads of newborn rats are illustrated. Although attempts were made to obtain true sagittal sections, the orientation cannot be considered microscopically perfect. For the following measurements sections were chosen which do not go through the midsagittal plane exactly, but show the nasopharyngeal duct open in its entire length. In these slightly parasagittal sections the upper incisor teeth are seen in *A* to *C*, but not in *D*. Since specimen *D* had actually no upper incisors, such parasagittal sections are somewhat more instructive than midsagittal sections.

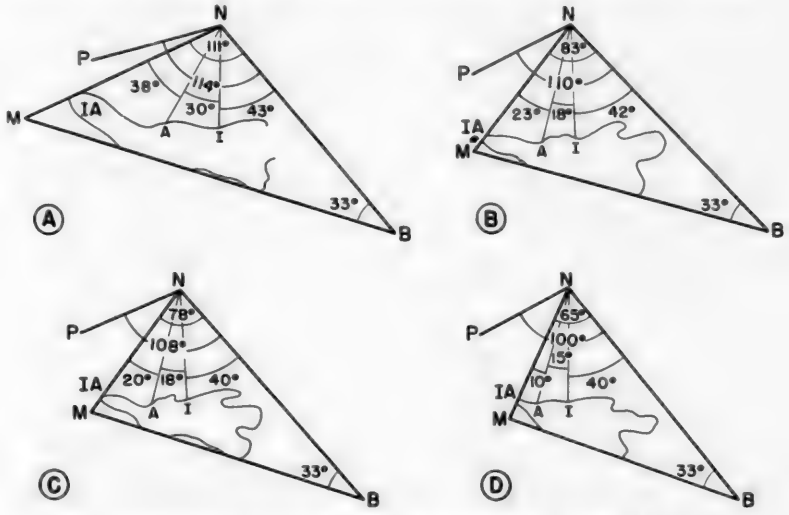


Fig. 3.—Craniofacial angles constructed on the basis of Fig. 2, *A* to *D*.

In the control (Fig. 4, *A*) oblique sections through the upper and lower incisors are seen. The upper incisor is surrounded by osseous premaxillary tissue and the lower incisor completely encased in the osseous mandible. The nasopharyngeal duct is separated from the oral cavity by the hard and soft palates. The tongue is well within the oral cavity. In the abnormal animal *B* the premaxillary area shows definite shortening and the section of the upper incisor tooth is changed in outline. This is due to an altered dentoalveolar relationship. A slight retrognathia of the mandible can be noticed and the lower incisor seen is small and shows evidence of malformations. The osseous structure of the mandible is hypoplastic. The tongue protrudes between the lips. Fig. 4, *C* shows little further change except a decrease of mandibular development. No lower incisor tooth is present and the mandible is reduced in height. In Fig. 4, *D* the premaxillary region is greatly underdeveloped and does not contain an incisor tooth. There is also a marked reduction in the soft tissues of this area, which results in vertical shortening

Fig. 4.—Sagittal sections through heads of newborn rats. *A*, Control; *B* to *D*, varying degrees of abnormality.

of the upper lip. This difference can be seen only by a comparison of Fig. 1, *A* and *B*. The lower jaw in Fig. 4, *D* is reduced in size like that of specimen in Fig. 4, *C* and the tongue protrudes beyond the rima oris.

In Fig. 5 the facial angles drawn upon the sections are illustrated. In these diagrams P represents prothion, N nasion, B basion, and IA the inferior alveolar point. Fig. 5, *A* shows the facial angles of the control animal and Fig. 5, *B*, *C*, and *D* the facial angles of the abnormal animals. In the control animal *A*, angle IANB is 70 degrees, The corresponding angle in the abnormal animal *B* is 67 degrees, in the abnormal animals *C* and *D* it is 50 degrees. The maximal loss of angle IANB in the series of abnormal animals is 20 degrees or 29 per cent. The other facial angle PNB is 108 degrees in the control animal (Fig. 5, *A*); it is 95 degrees in the abnormal animal *B*, 95 degrees in *C* and 93 degrees in *D*. The maximal loss of this angle in the series of abnormals is 15 degrees or 14 per cent.

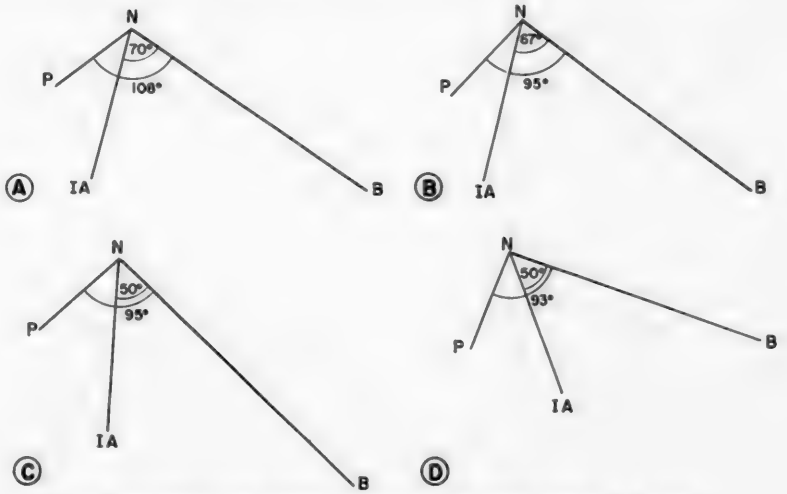


Fig. 5.—Facial angles constructed on the basis of Fig. 4, *A* to *D*.

DISCUSSION

The congenital facial anomalies described above illustrate that a characteristic pattern of osseous deformities may appear in the young of mothers suffering from a nutritional deficiency. Such results can be obtained only under striet experimental conditions in animals subjected to environmental regulations showing little resemblance to human living conditions. The fact that an experimental method results in the production of certain malformations in animals does not permit the conclusion that similar defects in man are due to similar causes. In fact, congenital dentofacial anomalies can be induced now in mammals by a variety of experimental methods;[9-17] they can be obtained also without experimentation by breeding animals with abnormal genetic endowment.[18, 19] It is very likely that comparable anomalies occurring

in man are also attributable to a variety of factors rather than to a single cause. It should be recognized that abnormal prenatal development may be due to the abnormal genetic constitution of the organism in some cases and due to prenatal environmental changes in others. Congenital malformations should be considered as symptoms of prenatal pathologic changes and not as etiologic entities. This concept is probably the most important result of the teratologic experiments performed in mammals in recent years. If this principle is clearly understood, certain aspects of animal experiments can be used to illustrate the mechanisms which lead to congenital malformations in man.

In the present experiments the entire embryo develops throughout gestation in an abnormal chemical environment created by a dietary deficiency of the mother. It is of interest that under such experimental conditions the skeleton is chiefly affected and that different parts of it suffer to a different degree. The axial skeleton and proximal parts of the appendicular skeleton are much less modified than distal structures.[3]

The angular relationships are partly changed and partly unaffected by the experimental conditions. Angle PNB, the facial angle which characterizes the position of the prothion, is only moderately reduced by the retarding process. In all the deficient young described the maximal reduction is found in angle MNA, which indicates that the premolar mandible is most severely affected. In the rat the bone about the lower incisor teeth may be looked upon as alveolar process. If this is true, the great reduction in mandibular length is partly due to failure of development of alveolar process. As indicated by angle ANI the molar corpus is markedly though less reduced than the premolar corpus. In contrast, angle INB is hardly affected by the deficiency. This fact, as well as histologic studies,[4] indicates that the ramus is almost normally developed in the anteroposterior direction. Angle NBM which represents the relationship of the cranial base to the inferior border of the mandible also remains unchanged in the abnormal young. This finding indicates normal vertical development of the ramus.

Such differential susceptibility of certain skeletal parts to a chronic nutritional deficiency to which the embryo is subjected throughout the gestational period is not easily explained. Acute teratogenic experiments which injure the developing embryo at critical periods result in specific malformations because the insult interferes with differentiation of parts which are rapidly growing at the time of injury. Parts which are at rest at this time are spared.[20] The specific effects of chronic anomalies of the environment can be explained only by the assumption that during certain phases of development the affected areas have special nutritional requirements which are not met under the experimental conditions. In this respect—and in this respect only—the experiments of nutritional deficiency resemble genetic deficiencies. An abnormal gene stays with the organism throughout life, but it manifests itself at critical periods only.[5]

Disproportional development of the components of the facial skeleton often is a major causative factor in the production of malocclusion. In the

experimental animals the reduction in size of the maxilla and the mandible are not proportionate. Therefore, malocclusion must be expected. If it is permissible to classify the malrelationship of the jaws and the "malocclusion" of the unerupted teeth in the deficient neonates using Angle's[21] human classification, it might be said that they resemble those of Class II malocclusion. The mandibular arch is in a posterior relationship to the maxillary arch. The lower molar teeth and the upper incisors which are all unerupted also show a position posterior to normal.

It should be of interest to the orthodontist that such severe dentofacial malrelationships can be induced by prenatal environmental factors. The malocclusions seen in later life and often referred to postnatal factors may actually originate in prenatal life.

SUMMARY

Congenital dentofacial malformations induced in rats by a maternal nutritional deficiency have been described. The resulting malrelationships and "malocclusions" have been evaluated by cephalometric methods. It was found that the facial angles were not equally affected by a changed prenatal chemical environment and that different parts of the facial skeleton were retarded disproportionately. Since the corpus of the mandible was more reduced than the maxilla, prerequisites for malocclusion were present.

REFERENCES

1. Warkany, J., and Nelson, R. C.: Skeletal Abnormalities in the Offspring of Rats Reared on Deficient Diets, *Anat. Rec.* **79**: 83, 1941.
2. Warkany, J., Nelson, R. C., and Schraffenberger, E.: Congenital Malformations Induced in Rats by Maternal Nutritional Deficiency. IV. Cleft Palate, *Am. J. Dis. Child.* **65**: 882, 1943.
3. Warkany, J., and Schraffenberger, E.: Congenital Malformations Induced in Rats by Maternal Nutritional Deficiency. VI. The Preventive Factor, *J. Nutrition* **27**: 477, 1944.
4. Warkany, J., and Deuschle, F. M.: Congenital Malformations Induced in Rats by Maternal Riboflavin Deficiency. Dento-facial Changes, *J. A. D. A.* **51**: 139, 1955.
5. Warkany, J.: Congenital Malformations Induced by Maternal Dietary Deficiency—Experiments and Their Interpretation, The Harvey Lectures, *series 48;* New York, 1952-53, Academic Press, Inc.
6. Dawson, A. B.: Note on the Staining of the Skeleton of Cleared Specimens With Alizarin Red S, *Stain Technol.* **1**: 123, 1926.
7. Margolis, H. I.: A Basic Facial Pattern and Its Application in Clinical Orthodontia, *Am. J. Orthodont. & Oral Surg.* **33**: 631, 1947.
8. Broadbent, B. H.: A New X-Ray Technique and Its Application to Orthodontia, *Angle Orthodontist* **1**: 45, 1931.
9. Warkany, J., and Schraffenberger, E.: Congenital Malformations Induced in Rats by Roentgen Rays, *Am. J. Roentgenol.* **57**: 455, 1947.
10. Wilson, J. G., Jordan, H. C., and Brent, R. L.: Effects of Irradiation on Embryonic Development. II. X-Rays on the Ninth Day of Gestation in the Rat, *Am. J. Anat.* **92**: 153, 1953.
11. Russel, L. B., and Russel, W. L.: An Analysis of the Changing Radiation Response of the Developing Mouse Embryo, *J. Cell & Comp. Physiol.* Suppl. 1, **43**: 103, 1954.
12. Fraser, F. C., and Fainstat, T. D.: Production of Congenital Defects in the Offspring of Pregnant Mice Treated With Cortisone, *Pediatrics* **8**: 527, 1951.
13. Ingalls, T. H., Curley, J., and Prindle, R.: Medical Progress: Experimental Production of Congenital Anomalies, *New England J. Med.* **247**: 758, 1952.
14. Gillman, J., Gilbert, C., and Gillman, T.: A Preliminary Report on Hydrocephalus, Spina Bifida, and Other Congenital Anomalies in the Rat Produced by Trypan Blue, *South African J. Med. Sc.* **13**: 47, 1948.

15. Giroud, A., and Boisselot, J. I.: Influence tératogène de la carence en acide folique, *Compt. rend Soc. de biol., Paris* **145**: 526, 1951.
16. Nelson, M. M., Asling, C. W., and Evans, H. M.: Production of Multiple Congenital Abnormalities in Young by Maternal Pteroylglutamic Acid Deficiency During Gestation, *J. Nutrition* **48**: 61, 1952.
17. Cheng, D. W., and Thomas, B. H.: Relationship of Time of Therapy to Teratogeny in Maternal Avitaminosis E, *Proc. Iowa Acad. Sc.* **60**: 290, 1953.
18. Grüneberg, H.: The Genetics of the Mouse, ed. 2, The Hague, 1952, Martinus Nijhoff.
19. Gluecksohn-Waelsch, S.: Some Genetic Aspects of Development, *Cold Spring Harbor Symposia on Quantitative Biol.* **19**: 41, 1954.
20. Stockard, C. R.: Developmental Rate and Structural Expression: An Experimental Study of Twins, "Double Monsters" and Single Deformities, *Am. J. Anat.* **28**: 115, 1921.
21. Angle, E. H.: Classification of Malocclusion, *D. Cosmos* **41**: 248 and 350, 1899.

THE PATHOGENESIS OF ABNORMAL ENAMEL FORMATION IN RABBITS INOCULATED WITH VACCINIA

SEYMOUR J. KRESHOVER AND JAMES A. HANCOCK, JR.

The Medical College of Virginia, School of Dentistry, Richmond, Va.

A PREVIOUS study demonstrated that marked abnormalities of odontogenesis may be manifested in rabbits following inoculation with vaccinia virus.[1] Although most of these animals developed a generalized infection shortly after intravenous injection of dermal lymph strain virus suspensions ranging in titer from 10^{-4} to 10^{-6}, no evidence could be found of infection involving the formative dental tissues. In the face of such negative findings, supported by the absence of Guarnieri bodies in sections through the apical region of incisor, premolar, and molar teeth, it was hypothesized that the disturbances in dental development may have been due to certain metabolic dysfunctions incident to the disease rather than to infected tissues per se. The peculiar, wavelike pattern of the observed dental defects further suggested their relationship to an altered rate of eruption.

It is of interest to note the remarkably similar picture of abnormal odontogenesis in the incisor teeth of rats following hypophysectomy,[2, 3] parathyroid dysfunction,[4] and magnesium deficiency.[5] Although Schour and Van Dyke[3] in their study of hypopituitarism suggested that continued dentin formation at the basal end of the incisors, coincident with arrested or retarded eruption, accounted for the so-called apical folding, Baume, Becks, and Evans[2] attributed this type of defect to an initial enamel aplasia and dentin hypoplasia which produced an area of weakness that readily buckled under the stresses of mastication.

Lending further difficulty to an understanding of the pathogenesis of this unique type of wavelike abnormality is our rather limited knowledge of the mechanism of tooth eruption. It is apparent that little additional information has been forthcoming from the many, and often contradictory, theories that have been proposed.[6]

On the basis of the character of the dental abnormality associated with vaccinia in rabbits and its striking resemblance to incisor defects in rats following magnesium deficiency and pituitary and parathyroid insufficiency, a further study of pathogenesis seemed advisable.

MATERIALS AND METHODS

A total of 19 rabbits comprised the material for this study. Table I presents their age, sex, and strain distribution. Eight served as controls and

This investigation was supported by a research grant from the United States Public Health Service.

Received for publication April 30, 1955.

TABLE I

RABBIT NO.		SEX	AGE (MONTHS)	STRAIN	CATEGORY	MAXILLARY INCISOR ERUPTION		AVERAGE SERUM CALCIUM (MG./100 C.C.)	AVERAGE SERUM PHOSPHORUS (MG./100 C.C.)	MEAN FOR GROUP		
						RATE (μ/DAY)	DAYS OBSERVED*			ERUPTION (μ/DAY)	CALCIUM (MG./100 C.C.)	PHOSPHORUS (MG./100 C.C.)
RB	11	M	12	Chinchilla	Control	165	47	13.5	5.3			
RB	12	M	12	California	Control	106	47	13.4	4.8			
RB	13	M	12	New Zealand	Control	191	47	12.6	3.6			
CP	1†	M	12	New Zealand	Control	211	52	14.6	3.1			
H	7	F	8	California	Control	192	30	—	—			
H	113	F	14	New Zealand	Control	229	35	—	—			
CP	7	F	12	New Zealand	Control	205	40	15.4	3.8			
CP	4	F	10	New Zealand	Control	140	41	13.7	—	180	13.9	4.1
CP	1†	M	12	New Zealand	Inoculated	147	34	—	—			
CP	9	M	6	New Zealand	Inoculated	103	90	13.8	3.9			
RB	7	M	12	California	Inoculated	145	31	13.5	3.7			
RB	8	M	12	New Zealand	Inoculated	98	61	14.1	3.5			
RB	9	M	12	New Zealand	Inoculated	131	61	14.3	3.6			
RB	10	M	12	New Zealand	Inoculated	242	61	15.0	5.0	144	14.1	3.9
CP	8	M	7	New Zealand	Grossly hypoplastic	50	92	14.2	4.2			
CP	6	F	30	New Zealand	Grossly hypoplastic	76	86	15.0	3.6			
S	29	M	20	New Zealand	Grossly hypoplastic	92	81	—	—			
RB	14	M	12	New Zealand	Grossly hypoplastic	63	32	14.5	4.0			
H	105	F	12	New Zealand	Grossly hypoplastic	86	81	—	—	73	14.6	3.9

*In the case of inoculated rabbits, beginning day of observation ranged from three to twenty-four days post inoculation.
†Served as a control animal before inoculation. Due to many previous cardiac punctures during control period, blood was not withdrawn.

6 were inoculated intravenously with dermal lymph strain vaccinia in dosages previously demonstrated to cause a sublethal, generalized infection. One of the latter group served as a control animal prior to inoculation. As reported in a previous study,[1] occasional instances of dental hypoplasia are observed among rabbits not previously or currently exposed to vaccinial infection. Invariably, these animals show signs or have a past history of upper respiratory infection. Nevertheless, their gross and histopathologic picture is indistinguishable from that frequently observed in rabbits after a sufficient postvaccinial interval has elapsed. Inasmuch as these defects are apparently nonspecific in nature, it seemed advisable to include such animals in the present study. Five were selected for this purpose.

Measurements of maxillary incisor eruption rates were made on all animals in accordance with the generally accepted procedure of notching the labial tooth surface and periodically recording its distance from the free gingival margin. These observations were made over a period of up to 52 and 92 days, respectively, for the control and hypoplastic groups. The inoculated rabbits were likewise observed for a period of up to 90 days following administration of virus. In addition to the recording of eruption rates, a series of from 3 to 6 concurrent determinations of serum calcium and phosphorus were made on animals in each group. Serum calcium was measured by rapid titration with disodium dihydrogen ethylenediamine tetraacetate, as described by Elliott.[7] The method of Fiske and Subbarow[8] was used to determine the levels of serum phosphorus.

Following the outlined experimental procedure, the rabbits were sacrificed and radiographs taken of their jaws. Maxillae and mandibles were then prepared for histologic study by the double imbedding technic.

FINDINGS

It will be noted from Table I that although rather wide ranges of maxillary incisor eruption rates exist in each group, a comparison of their mean values shows rather significant differences. In the 8 control animals there was a range of from 0.106 to 0.229 mm. per day with no apparent correlation between the varying rates and the age, sex, or strain of the animal. Furthermore, the factors of diet, temperature, housing conditions, etc., were so standardized as to be considered of no significant influence. However, with a mean rate of 0.180 mm. per day and a standard deviation of ±0.040, it is evident that marked differences exist between individual rabbits.

A similar wide range was observed in the group of 6 vaccinia inoculated animals which had a mean eruption rate of 0.144 mm. per day and a standard deviation of ±0.052. Despite the rather marked variability of eruption, these data suggest a differential trend between the inoculated and control groups ($P = 0.2 < 0.1$). Although the dental abnormalities in the inoculated group were oftentimes manifested in radiographs, the time intervals between inoculation and sacrifice were not sufficiently long to allow for the development of clinically demonstrable defects. A previous publication[1] demonstrated the

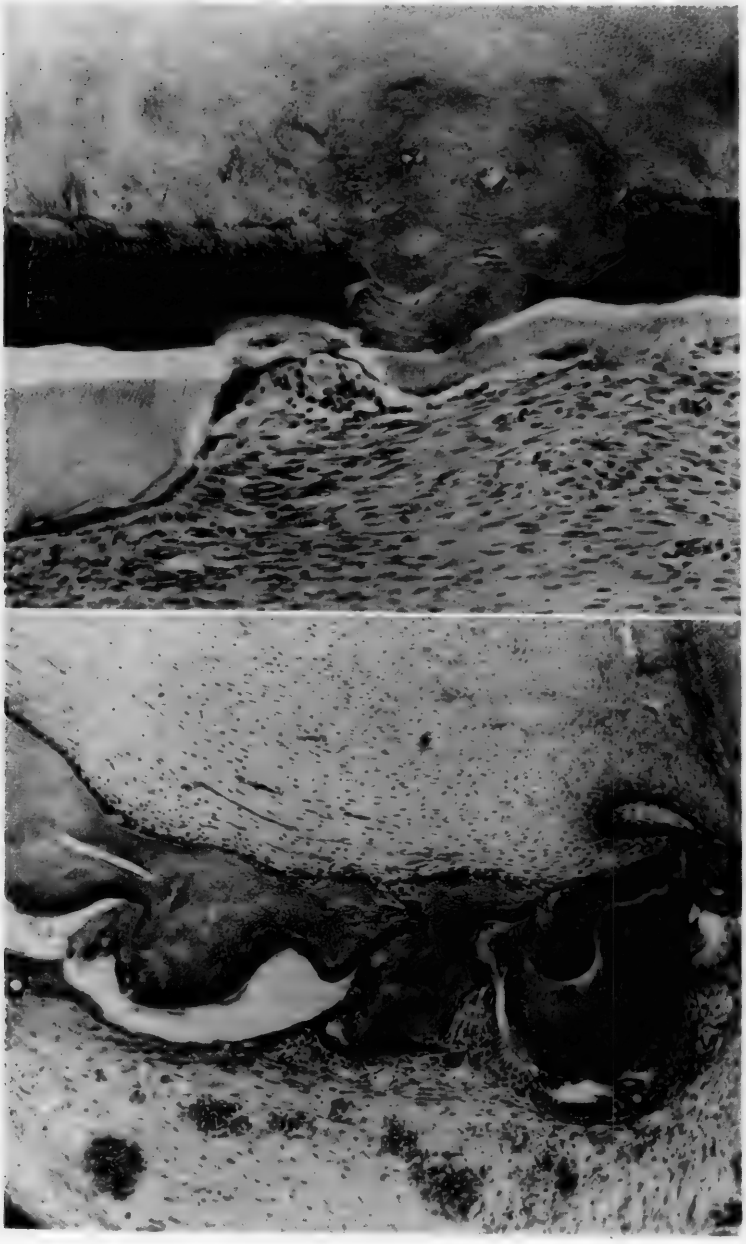
extensive deformity that could appear on the clinical crowns of incisor teeth after a sufficiently long postinoculation period. A comparison between a similar gross defect and the corresponding teeth of a control animal is shown in Fig. 1. In contrast to these two extremes, the recently inoculated group showed a striking but somewhat less severe hypoplasia which was limited in extent to the basal ends of involved teeth. However, as the postinoculation time interval lengthens, such defects become more apparent and extensive (Fig. 2).

A. *B.*

Fig. 1.—*A*, Incisor teeth of a control rabbit. *B*, Marked horizontal linear grooving of incisor teeth of rabbit inoculated with vaccinia virus 329 days previously.

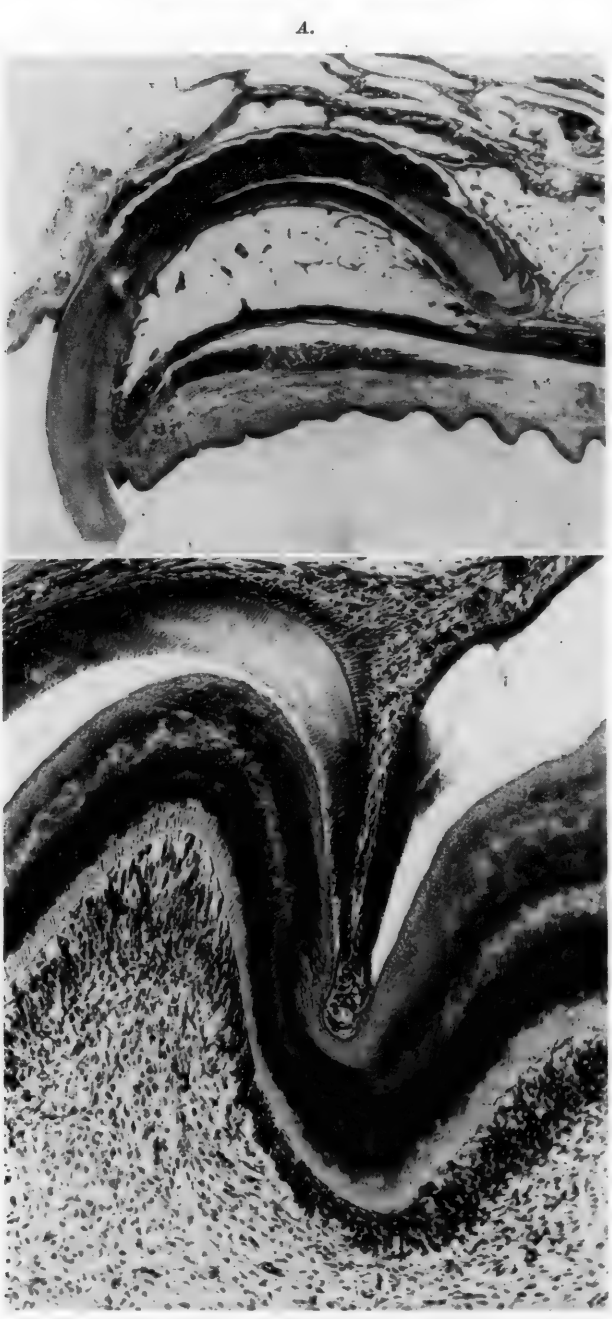
Although only 5 rabbits comprised the category with gross dental hypoplasias, they manifested a striking retardation of eruption rate when compared to the control group ($P = 0.001$). With a mean eruption rate of 0.073 mm. per day, the sequence of events in the pathogenesis of the specific type of dental defect under study becomes somewhat more clarified; namely, a progressive slowing of tooth eruption with a consequent buckling or folding of their basal ends (Fig. 3).

In order to evaluate parathyroid function as a possible etiologic factor in dental abnormalities following vaccinia virus inoculation, a series of serum calcium and phosphorus determinations were made. The mean levels in control animals were 13.9 and 4.1 mg. per cent, respectively. These figures compared quite well with the 14.1 and 3.9 mg. per cent values obtained in the inoculated group. The calcium (14.6) and phosphorus (3.9) levels noted in the hypoplastic rabbits are not significantly different from either of the other groups.



A. *B.*

Fig. 2.—*A*, Maxillary incisor of a rabbit inoculated with vaccinia virus 100 days prior to sacrifice. Dentin and enamel in basal region are markedly folded in appearance with occasional areas of complete enamel arrest. *B*, Area of enamel aplasia and abnormal dentin formation in basal region of right maxillary second premolar of rabbit inoculated with vaccinia virus 100 days prior to sacrifice. A prominent incremental line separates the dentin into 2 layers differentiated chiefly by change in tubular direction. The dentin proximating pale-staining enamel matrix is quite irregular and shows osteoid-like pattern in region adjacent to zone of enamel aplasia.



A.

B.

Fig. 3.—*A*, Maxillary incisor of a rabbit inoculated 196 days prior to sacrifice. Wavelike pattern of enamel matrix and dentino-enamel junction is particularly marked in apical third. *B*, High magnification through apical region of same tooth. Enamel matrix gradually thins as it extends into depth of crevice so that it is ultimately lost. Underlying dentin is globular and characterized by prominent, dark and light staining, incremental markings.

DISCUSSION

From the limited data presented, it is suggested that the peculiar nature of the described developmental dental disturbances may be explained by a progressive slowing of the eruption rate. It is further evident that the abnormalities of enamel and dentin matrix formation are not related to an altered calcium and phosphorus metabolism nor do matrix disturbances seem to be the mechanism by which apical folding occurs. However, it is likely that the pathogenesis of such defects may be quite variable, as indicated by the almost identical findings following hypophysectomy,[2, 3] parathyroidectomy,[4] and magnesium deficiency.[5] Certainly, no evidence is yet available that a common pathway of endocrine or other dysfunction exists in all such cases. Nevertheless, the clinical and histopathologic findings in the present study would seem to support the suggestion of Schour and Van Dyke[3] that continued dentin formation at the basal ends of the incisors, coincident with retarded eruption, is responsible for the general type of dental deformity under consideration. Less evidence is available to attribute the defects to sites of weakened aplastic enamel and dentin that buckle under the stresses of mastication, as reported by Baume, Becks, and Evans[2] in their study of magnesium deficient rats.

SUMMARY AND CONCLUSIONS

Marked developmental dental defects may be noted in rabbits that have been infected with vaccinia virus. In an attempt to clarify the pathogenesis of such disturbances, eruption rates, as well as a number of calcium and phosphorus determinations, were made on 8 control, 6 recently inoculated, and 4 grossly hypoplastic rabbits. It was observed that eruption is significantly slowed after inoculation and even more so retarded at the time hypoplastic lesions appear on the clinical crowns. No significant departures from control levels were noted in the blood serum calcium and phosphorus readings of either experimental group.

Although the dental abnormalities following vaccinia inoculation are quite identical to those described in parathyroidectomy, hypophysectomy, and magnesium-deficient rats, it is difficult to conceive of a common denominator to explain the pathogenesis in all cases. Nevertheless, they all have in common a retarded rate of eruption which seems to be the principal mechanism by which the wavelike apical deformities are produced.

REFERENCES

1. Kreshover, S., Clough, O., and Hancock, J.: Vaccinia Infection in Pregnant Rabbits and Its Effect on Maternal and Fetal Dental Tissues, *J. A. D. A.* **49**: 549, 1954.
2. Baume, L., Becks, H., and Evans, H.: Hormonal Control of Tooth Eruption. II. The Effects of Hypophysectomy on the Upper Rat Incisor Following Progressively Longer Intervals, *J. D. Res.* **33**: 91, 1954.
3. Schour, I., and Van Dyke, H.: Changes in the Teeth Following Hypophysectomy. I. Changes in the Incisor of the White Rat, *Am. J. Anat.* **50**: 397, 1932.

4. Schour, I., Chandler, S., and Tweedy, W.: Changes in the Teeth Following Parathyroidectomy. I. The Effects of Different Periods of Survival, Fasting and Repeated Pregnancies and Lactations on the Incisor of the Rat, *Am. J. Path.* **13**: 945, 1937.
5. Becks, H., and Furata, W.: Effect of Magnesium Deficient Diets on Oral and Dental Tissues. II. Changes in the Enamel Structure. *J. A. D. A.* **28**: 1083, 1941.
6. Massler, M., and Schour, I.: Studies in Tooth Development: Theories of Eruption, *Am. J. Orthodontics & Oral Surg.* **27**: 552, 1941.
7. Elliott, W.: Volumetric Determination of Calcium in Blood Serum. *J. Biol. Chem.* **197**: 641, 1952.
8. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, *J. Biol. Chem.* **66**: 375, 1925.

THE EFFECTS OF ALUMINUM OXIDE ON THE LUNGS OF MICE

CHARLES A. CLARK, M.Sc., D.D.S.*

College of Dentistry, Howard University, Washington, D. C.

THE extent of damage that the "dust" diseases do to the lungs is due to the chemical or physical nature of the "dust" and the duration of exposure. The long-continued inhalation of certain irritating dusts may cause a chronic interstitial reaction known as pneumoconiosis. Since a "dust" is associated with the use of the airbrasive technic, the possibility of pneumoconiosis from its use has been suggested. This study is concerned with the possible lung damage from the "dust" associated with this dental procedure.

Fullmer and Eastman[6] reported the effects of aluminum oxide on the lungs of rabbits as a weak chronic granulation reaction with many macrophages, a few foreign body giant cells, and slight fibrous tissue proliferation. Their experiment ran from 6 weeks to 17 months. The method described by King, Clegg, and Rae,[5] in which the aluminum oxide was suspended in normal saline and, by use of a small catheter, forced into the lungs of the rabbits once per month, was used.

Kerr, Ramfjord, and Ramfjord[4] developed methods of inhalation of air-brasive powder using rabbits, guinea pigs, and monkeys. The exposure time was 13 to 222 days and the animals were sacrificed 15 to 648 days after the initial exposure. They found atelectasis, emphysema, mild patchy fibrosis, and the accumulation of phagocytized particles in the perivascular tissues and the peribronchial lymph nodes.

MATERIALS AND METHODS

Thirty white mice were used. Fifteen mice, selected at random, were kept in ¼ inch mesh wire cages, 1 foot in diameter. Each cage was placed in a cardboard box just large enough to accommodate it. At one end of the box, a hole was cut to insert the head of the vacuum-recovery unit of the machine and it was sealed with masking tape. At the other end, another hole of 1 inch diameter was placed. Cavity preparations were made on extracted teeth 1 inch from the 1 inch hole of the box. When the vacuum was turned on, a mixture of aluminum oxide and tooth material passed through the cages containing the mice. This was done for periods of 15 minutes once per day for 5 weeks.

As controls, 15 mice were kept under identical conditions but extracted teeth were cut with green stones using a rotary dental engine. This, too, was done 1 inch from the 1 inch hole with the vacuum turned on, insuring circulation of the ground tooth material and any material from the stones.

Received for publication June 5, 1955, revised by author May 22, 1956.

*Present address: Medical Center, Jersey City, N. J.

Three mice of each group were sacrificed with chloroform immediately after the experiment ended. The lungs and bronchi were removed and immediately fixed in 10 per cent formalin. The sections were stained with hematoxylin and eosin.

Subcutaneous injections of aluminum oxide were done on 1 mouse that had no experience with the material. One-half cubic centimeter of a suspension of 1 gram aluminum oxide per 1 c.c. of normal saline was injected. After 2 weeks, biopsies were performed and sections made.

Microscopic preparations were made of blood and the lungs of control mice. Before staining, the powdered aluminum oxide was sprayed on the slides for study of the comparative size of the particles.

RESULTS

The lungs of the 3 mice exposed to the aluminum oxide by inhalation and sacrificed at the end of the experiment showed typical bronchopneumonia, with congestion of the blood vessels of the alveolar walls, massive infiltration by polymorphonuclear leukocytes, lymphocytes, monocytes, and accumulation of tissue fluids. There was complete consolidation in certain areas of some of the lobes. No noteworthy change took place in the control mice.

Two more experimental mice were sacrificed 2 weeks later and sections of both showed the same type of reaction as the above.

During the course of the experiment, 9 of the mice which received the aluminum oxide died. Only 1 of the control mice died. Unfortunately, due to a gross oversight, these animals were not autopsied.

In the biopsy specimen of the injected "dust" suspension, it was noted that encapsulation of the material had occurred after a period of 2 weeks. Upon microscopic examination, a typical foreign body reaction was observed with the production of foreign body giant cells. The particles of aluminum oxide sprayed on the slides were as large or larger than some alveolar spaces.

DISCUSSION AND SUMMARY

A method was developed whereby mice received aluminum oxide and tooth material through inhalation. During the course of the experiment, 9 of the mice which received the aluminum oxide died, while only 1 of the control group died.

Three mice and their control mice were sacrificed after 5 weeks of experimentation. Upon microscopic examination, the control mice showed no observable changes. Those which received the aluminum oxide showed the microscopic picture of bronchopneumonia with patchy consolidation of a lobe or part of it due to congestion, edema, and massive infiltration of polymorphonuclear leukocytes, lymphocytes, and monocytes. Two experimental mice which were sacrificed 2 weeks later, after having been exposed to the dust for 5 weeks, showed the same general reaction.

The stage of early parenchymatous lesions of Klotz's[2] classification of pneumoconiosis has been demonstrated experimentally.

None of the particles could be demonstrated in the lung fields, including the consolidated areas, of any of the animals that developed pneumonia. This, of course, raised the question as to the disposition of the particles. For that reason subcutaneous injections of saline suspensions of aluminum oxide were made. After 2 weeks, biopsies were performed and the areas were found to be encapsulated and liberally interspaced with giant cells, but the integrity of the particles remained the same. This indicated a lack of dissolution of the particles by the living tissue.

Aluminum oxide was sprayed on the slide of a control animal's lung. The particles were as large, and in some cases larger than the alveolar spaces.

Though problematical, the evidence seems to indicate that an inflammatory reaction occurred distal to the point of contact of the particles which were eliminated by the ciliary activity of the epithelium.

The author wishes to extend his appreciation to Drs. R. Jason, and R. Hayes for aid in microscopic interpretations, to Drs. W. Booker, and H. Gaskins, for suggestions, and to Mr. Murdock for help in the preparations of slides and photomicrographs.

REFERENCES

1. Kerr, D. A., Ramfjord, S., and Ramfjord, G. M.: Effects of Inhalation of Airbrasive Powder, *J. D. Res.* **33**: 666, 1954.
2. Klotz, O.: Progress of Medical Science, *Am. J. M. Sc.* **188**: 418, 1934.
3. Bell, E. T.: Textbook of Pathology, ed. 6, Philadelphia, 1949, Lea & Febiger, p. 129.
4. Boyd, W. A.: Textbook of Pathology, ed. 5, Philadelphia, 1952, Lea & Febiger, p. 437.
5. King, E., Clegg, J., and Rae, V.: The Effects of Asbestos and Aluminum on the Lungs of Rabbits, Thorax **1**: 188, 1946.
6. Fullmer, W., and Eastman, R.: Effects of Aluminum Oxide on the Rabbit Lung, *J. D. Res.* **31**: 487, 1952.

A STUDY OF INFERIOR ALVEOLAR NERVE BLOCK ANESTHESIA IN HUMANS COMPARING 2.0 PER CENT PROCAINE WITH 2.0 PER CENT PROCAINE PLUS 1:50,000 EPINEPHRINE

EMMETT R. COSTICH, D.D.S., M.S., PH.D.*

From the Department of Dentistry, University of Rochester, School of Medicine and Dentistry, and The Eastman Dental Dispensary, Rochester, N. Y.

IT IS the accepted practice in the preparation of local anesthetics for dental surgery to use a 2.0 per cent procaine solution containing epinephrine in a concentration of 1:50,000. This combination of agents is employed to maintain a longer period of anesthesia; without some adjunct to produce vasoconstriction, the action of the anesthetic may be only transitory.[1] From many studies in which intradermal injections of procaine were made with and without epinephrine, it has been the general conclusion that the duration of anesthesia without epinephrine is approximately eighteen to twenty minutes, while with epinephrine it varies from sixty to ninety minutes. Block anesthesia using a solution containing no epinephrine has been reported[2] to be of longer duration than an infiltration type of anesthesia of which the intradermal wheal is an example. No supporting data on this subject could be found in the literature. It therefore seemed advisable to carry out a study to determine the quality, duration, and reaction of nerve block anesthesia produced in human beings by solutions of 2.0 per cent procaine plus 1:50,000 epinephrine, and 2.0 per cent procaine containing no epinephrine.†

METHOD

The use of patients presenting themselves for dental surgery in a hospital clinic was contemplated but rejected for several reasons: emotional strain from worry about the pending surgery; variable age, health, and educational backgrounds; and because it has been found advisable to disregard initial visits when carrying out such studies.[3]

Fifty medical students, 40 males and 10 females, 22 to 32 years of age served as experimental subjects. Each received 5 inferior alveolar block injections at approximately weekly intervals. The initial injection served only as training and was disregarded in tabulating data. The remaining 4 injections consisted of 2 with 2.0 per cent procaine and 1:50,000 epinephrine and 2 with 2.0 per cent procaine alone.

This work was supported in part by a grant from the Medical Research and Development Board, Office of the Surgeon General, Department of the Army (Contract No. W 49-007-MD-420) and is based, in part, upon the thesis of the author for the degree of Master of Science in Pathology, June, 1949.

Presented before the 27th General Meeting of the International Association for Dental Research, Chicago, Ill., June 24 to 26, 1949. (J.D.R. 28: 637, 1949.)

Received for publication June 8, 1955.

*Present address: School of Dentistry, University of Michigan.

†The procaine-epinephrine and plain procaine solutions used were generously supplied by the Cook-Waite Laboratories, Inc., New York, N. Y.

The students were not informed as to which solution was given and the order was varied with different subjects. The volume of solution used was 2 ml. with an injection time of twenty to thirty seconds. All injections were made on the right side to maintain the same injection technic.

The pulse, blood pressure, and respiration were recorded before injection and 2 minutes after injection. The onset time for anesthesia was recorded as the time elapsed between completion of the injection and the first sign of anesthesia as reported by the subject.

When the study was begun, a condenser discharge apparatus was used to determine the onset of anesthesia.[3] This was abandoned after the first series of injections were completed because the subjects were all aware of beginning anesthesia about fifteen to twenty seconds before the electrical stimuli disappeared. However, the use of a condenser discharge type stimulator may be a good method for testing the onset of anesthesia in a less intelligent or uncooperative group of people.

At the time the subjects appeared for their injections, they were observed for signs of sweating, tremors, or faintness so that a base could be established for determining any increase in these signs which might follow the injections.

The subjects were dismissed about ten minutes following the injection and asked to note the time when the anesthesia began to fade; not the time when anesthesia completely disappeared. This method of recording duration was chosen because it was felt it gave a more accurate record of the effective time during which surgery could be performed.

Besides noting the duration of anesthesia, it was also requested that the patient note any postanesthesia symptoms and the duration of such symptoms.

RESULTS

Because the group of subjects was not limited to one sex, the findings were tabulated separately to see if there was any variation in response which might be attributed to sex differences. There was only one difference found between males and females; the females showed no differences in onset time between the 2 solutions, whereas the males showed a significant difference. The lack of difference in the females could be accounted for by abnormally long onset times in 2 individuals receiving plain procaine, which would make a great difference in such a small sample (10 females as compared to 40 males). Therefore the results are reported on the experimental group as a unit and not according to sex.

The comparison of pre- and postinjection findings for pulse, blood pressure, and respiration showed a variety of responses. Some individuals were consistent in their responses to the 2 solutions while others gave a different set of responses to each injection.

It can be seen from Table I that the tendency was for pulse rate to increase with procaine-epinephrine and to decrease with plain procaine. The systolic and diastolic blood pressure dropped with both solutions but the change was

TABLE I

MEAN VARIATION OF PULSE, BLOOD PRESSURE, AND RESPIRATION FOLLOWING INJECTION

		PULSE			SYSTOLIC PRESSURE			DIASTOLIC PRESSURE			PULSE PRESSURE			RESPIRATION		
		NO. CASES	%	MEAN CHANGE	NO. CASES	%	MEAN CHANGE	NO. CASES	%	MEAN CHANGE	NO. CASES	%	MEAN CHANGE	NO. CASES	%	MEAN CHANGE
Procaine 2% Plus Epinephrine 1:50,000	*Rise*	48	50	+10	36	38	+6	19	20	+7	46	48	+10	19	20	+4
	Fall	25	26	−9	54	56	−8	62	64	−8	39	41	−7	4	4	−4
	No Change	23	24	—	6	6	—	15	16	—	11	11	—	73	76	—
Procaine 2%	*Rise*	35	38	+8	20	22	+6	33	36	+5	30	30	+6	12	13	+4
	Fall	39	42	−10	54	59	−6	33	36	−6	53	57	−9	8	9	−4
	No Change	18	20	—	18	19	—	26	28	—	9	10	—	72	78	—

TABLE II

STATISTICAL ANALYSIS OF PRE- AND POSTINJECTION RECORDS OF PULSE, BLOOD PRESSURE, PULSE PRESSURE, AND RESPIRATION

		PROCAINE 2% PLUS EPINEPHRINE 1:50,000						PROCAINE 2%					
		NUMBER OF INJECTIONS	MEAN VALUE	STANDARD DEVIATION	M_1-M_2	t	p	NUMBER OF INJECTIONS	MEAN VALUE	STANDARD DEVIATION	M_1-M_2	t	p
Pulse	Before	96	78	11	4	1.63	.10	92	77	12	1	0.50	.61
	After	96	82	13				92	76	9			
Systolic Pressure	Before	96	118	11	3	1.34	.18	92	116	11	3	1.22	.23
	After	96	115	11				92	113	12			
Diastolic Pressure	Before	96	70	7	5	2.89	<.01	92	70	8	1	0.50	.61
	After	96	65	8				92	69	9			
Pulse Pressure	Before	96	47	8	2	1.00	.31	92	46	8	2	1.15	.25
	After	96	49	10				92	44	9			
Respiration	Before	96	20	2	0	....	....	92	20	2	0	....	....
	After	96	20	2				92	20	2			

greatest in the procaine-epinephrine group. Pulse pressure tended to rise in the procaine-epinephrine group and fall in the plain procaine group. Respiratory rate showed little change in either group.

The preinjection data for the 2 solutions compared favorably; there were no statistically significant differences. This served as a check on the technics of injection and observation. The statistical analysis of the postinjection figures (Table II) revealed no significant differences except in the case of the diastolic blood pressure with the procaine-epinephrine where there was a drop in the postinjection pressure. This observation is consistent with the findings of Starr, Gamble, Margolies, Donald, Joseph, and Eagle[4] from a study of the cardiovascular effects of epinephrine given subcutaneously.

TABLE III

STATISTICAL ANALYSIS OF TIME OF ONSET OF ANESTHESIA AND DURATION OF ANESTHESIA

	NUMBER OF INJECTIONS	MEAN VALUE	STANDARD DEVIATION	M_1-M_2	t	p
Onset in Seconds						
Procaine 2% Plus epinephrine 1:50,000	96*	184	195	51	2.35	.02
Procaine 2%	91†	133	70			
Duration in Minutes						
Procaine 2% Plus epinephrine 1:50,000	96‡	90	31	54	14.79	<.01
Procaine 2%	87§	36	14			

*Two patients failed to get anesthesia.
†Five patients failed to get anesthesia.
‡Two patients failed to have more than onset symptoms.
§Four patients failed to have more than onset symptoms.

The onset of anesthesia was found to be slightly faster with plain procaine (Table III). The time between injection and onset of anesthesia was one hundred thirty-three seconds for plain procaine and one hundred eighty-four seconds for procaine-epinephrine. This difference is not highly significant and is of no clinical importance since the mean difference is only fifty-one seconds. The similarity of onset time can be more clearly seen in the frequency distribution (Fig. 1) which shows 66 cases for each solution falling in the time range of sixty to one hundred eighty seconds.

The duration of anesthesia was longer with procaine-epinephrine than with plain procaine; there was a mean difference of fifty-four minutes (Table III). This difference was found to be highly significant, having a p value of less than 0.01. The frequency peak for plain procaine was forty minutes and that for procaine-epinephrine, ninety minutes (Fig. 2). This is of clinical importance where dental appointments are usually of an hour's duration.

There were 4 anesthetic failures with procaine-epinephrine and 9 with plain procaine. There were 3 patients who failed to get anesthesia on either of 2 occasions when given plain procaine solution. One of these individuals also failed

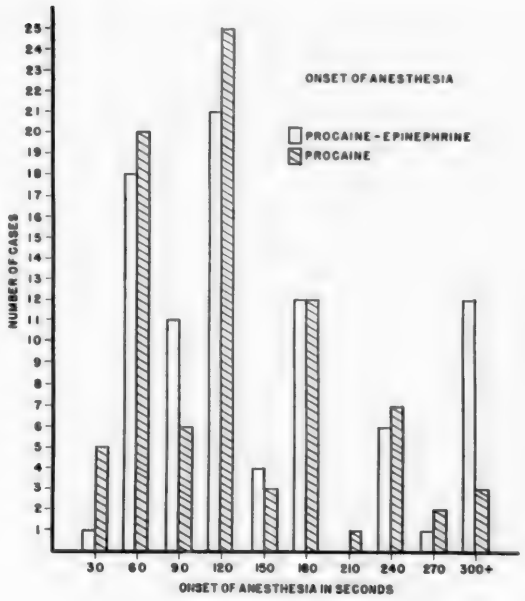

ONSET OF ANESTHESIA
PROCAINE - EPINEPHRINE
PROCAINE
NUMBER OF CASES
ONSET OF ANESTHESIA IN SECONDS
30 60 90 120 150 180 210 240 270 300+

Fig. 1.

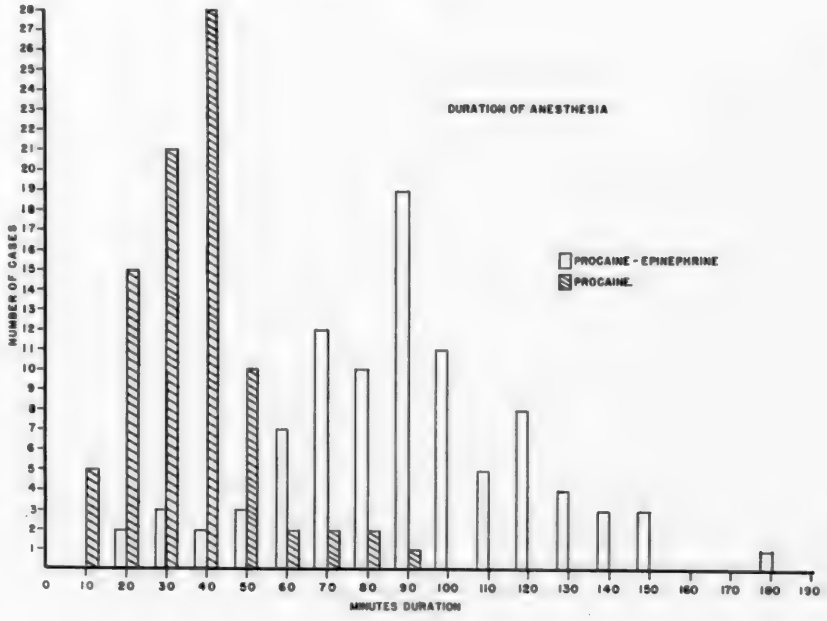

DURATION OF ANESTHESIA
PROCAINE - EPINEPHRINE
PROCAINE
NUMBER OF CASES
MINUTES DURATION
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190

Fig. 2.

to get anesthesia on 1 occasion when given procaine-epinephrine. The failures in these patients might be due (a) to rapid destruction of the procaine, (b) to an individual variation in procaine tolerance, or (c) to deposition of the solution at such a distance from the nerve trunk that the anesthetic was too dilute to exert an effective action by the time it had diffused to the trunk. The other individual failures probably fall into this latter category.

The untoward reactions of sweating, tremors, and faintness were arbitrarily graded as 1 plus for a mild reaction to 4 plus for a pronounced reaction. It can be seen from Table IV that there was a greater number of reactions in the group receiving the procaine-epinephrine. Statistical comparison of the number of patients showing reactions in the 2 groups by the chi square method gave a p value of less than 0.01, indicating that the difference is significant.

TABLE IV
SYSTEMATIC REACTIONS

	PROCAINE-EPINEPHRINE				PROCAINE			
Number injections	98				96			
Number patients showing reactions	15				2			
Reactions*	4+	3+	2+	1+	4+	3+	2+	1+
Tremor	2	2	7	1	0	0	1	0
Sweating	1	1	1	1	0	0	1	0
Faintness	0	1	1	1	0	0	0	1

*Several patients exhibited more than one type of response and in varying degrees.

The postinjection symptoms reported consisted of pain or stiffness. Almost all patients reported a slight stiffness, which lasted for a few hours after anesthesia and was not troublesome. However, 19 per cent of those receiving procaine-epinephrine reported pain or stiffness still present on the day following injection and 9 per cent of those receiving plain procaine reported similar symptoms. The difference is not statistically significant.

Each subject was asked to express an opinion on the quality of anesthesia without knowing which solution had been injected. Several of the patients who received the plain procaine experienced a very profound anesthesia which lasted about fifteen minutes and then rapidly faded. However, the majority of the group receiving plain procaine felt that the anesthesia was poor and preferred not to receive that solution for dental operations. The majority of subjects felt that the procaine-epinephrine gave a profound anesthesia and reported that they would not object to dental work under that type of anesthetic. A comparison of the replies to the question on quality of anesthesia was made by the chi square method and gave a p value of 0.01, indicating that the difference of opinion concerning anesthetic quality with these 2 types of solutions is significant.

CONCLUSIONS

One may conclude from this study that 2.0 per cent procaine without epinephrine, when given as an inferior alveolar nerve block, fails to produce anesthesia of sufficient depth or duration to warrant its routine use in dental operations.

The 2 per cent procaine solution containing epinephrine in a concentration of 1:50,000 gives adequate depth and duration of anesthesia but has the disadvantage of increasing the occurrence of undesirable side effects such as sweating, tremors, and faintness. Epinephrine may also play a part in the occurrence of postinjection pain and stiffness. The cause of this latter problem may be the result of irritation to the tissue by a combination of factors or a single factor such as:

A single constituent of the solution (procaine, epinephrine, bisulfite), breakdown products of the solution (paraaminobenzoic acid, diethylaminoethanol, adrenochrome, etc.), the low pH of the solution (3.3 to 5.5), prolonged retention in the injected area, or prolonged ischemia. Further studies of this nature with more dilute concentrations of epinephrine might aid in the establishment of a more desirable anesthetic solution.

SUMMARY

1. Fifty medical students received right inferior alveolar nerve blocks of 2.0 per cent procaine solution with and without 1:50,000 epinephrine.

2. The subjects were observed for changes in pulse rate, blood pressure, pulse pressure, respiratory rate; sign of tremor, sweating, and faintness; and onset, duration, and quality of anesthesia and postanesthetic pain.

3. Procaine-ephinephrine injection resulted in the following statistically significant differences as compared to plain procaine: (a) drop in postinjection diastolic blood pressure, (b) longer duration of anesthesia, (c) increased number of untoward reactions, and (d) more desirable depth of anesthesia.

REFERENCES

1. Sollmann, T.: Comparative Efficiency of Local Anesthetics. V. Anesthesia of Human Skin. General Conclusions, *J. Pharmacol. & Therap.* **11**: 69, 1918.
2. Gunter, J. H.: Notes on Local and General Anesthesia for Dental Students, Philadelphia, 1942, College Offset Press, p. 4.
3. Costich, E. R.: A Preliminary Study of the Efficiency of Piperocaine Hydrochloride (Metycaine) as a Local Anesthetic in Dental Surgery, *J. A. D. A.* **40**: 163, 1950.
4. Starr, I., Gamble, C. J., Margolies, A., Donal, J. S., Jr., Joseph, N., and Eagle, E.: Clinical Study of the Action of Ten Commonly Used Drugs on Cardiac Output, Work and Size; on Respiration, on Metabolic Rate and on the Electrocardiogram, *J. Clin. Invest.* **16**: 799, 1937.

THE INFLUENCE OF DESIGN FACTORS ON THE PERFORMANCE OF THE INVERTED CONE BUR

E. E. HENRY, PH.D.

School of Dentistry, University of Michigan, Ann Arbor, Mich.

INTRODUCTION

THE advent of cavity-shaping technics, which utilize high rotational speeds and a light hand pressure to remove a large amount of tooth material, has resulted in an acute awareness by dental operators that certain burs are superior to others. This situation has caused an obvious desire by the dentist, the dental researcher and the manufacturer for knowledge which will explain these differences in cutting ability. This report is a partial result of a program to determine the influence of various design factors on the performance of dental burs.

The actual cutting ability of a bur depends on two qualities, (1) the true cutting ability and (2) any clogging tendencies. This program was designed to study the first quality while eliminating the second as much as possible. For this reason the inverted cone bur with its short length of engagement has been chosen for specific study.

Dental bur manufacturers are faced with a very difficult problem; namely, the inexpensive production of a bur with keen sharp flutes. They can produce better burs than are available at the dental supply stores. However, the cost would be considerably higher than the present market price, so as to be economically unfeasible. This is particularly true in the production of steel burs. It is, therefore, the intent of this program to help them, in conjunction with these research departments, produce for the dental operator as good a bur as possible, while remaining within economic limits.

LITERATURE REVIEW AND PURPOSE

Henry and Peyton[1] have reported on the effect of variation in speed and pressure on the cutting ability of dental burs. We[2] have also compared burs by various manufacturers and related their findings to significant differences in bur design. Lammie[3] studied the rosehead or spherical bur constructed in tungsten carbide. Staunt[4] studied the cutting of enamel by tungsten carbide burs and diamond stones. One of the most enlightening advances in dental bur testing is the use of high speed photography by Hudson and Hartley[5] and their associates at the National Bureau of Standards to photograph a bur

This study was supported by a contract between the Office of the Surgeon General, United States Army, Contract No. DA-49-007-MD-141, and the University of Michigan.

Presented at the meeting of the Dental Materials Group, I.A.D.R., Chicago, March 18-20, 1955 (*J. D. Res.* **34**: 743, 1955).

Received for publication April 21, 1955.

during the actual cutting of tooth material. The photographs show that only a few bur flutes actually do the cutting and show very dramatically the effects of eccentricity in the bur shaft. Bryton, Skinner, Lindenmeyer, and Lasater[6] have studied bur design and reported on the use of various materials for testing purposes. They reported that ivorene, one of the materials used in this program, cuts 5 times as fast as dentin; without, however, any increase in the clogging tendency. They suggested the use of a "standard ivory" as a testing material.

Specifically, the purpose of this program is to study the effect of 6 variables on the cutting ability of the bur. These variables are: (1) the finish, (2) heat treatment, (3) end flutes, (4) changes in bur diameter, (5) changes in length of engagement, and (6) changes in the number of flutes.

THE EFFECT OF FINISH

The dental bur is formed by cutting each flute into the bur blank with a preformed rotating cutter that is progressing nearly parallel to the axis of the bur. Some machines cut the side and end flutes in one continuous operation while other machines only perform one operation; it being necessary to use 2 machines to cut both flutes. During the first cut or pass of the cutter the flute is roughly formed. The second cut places a cutting edge on the bur flute. However, considerable roughness along the flute either in the form of depressions ("nicks") or excess material ("fins") still remains. This excess material may be removed by making subsequent passes or cuts of the bur flutes. It is said that the fins break off the first time that the bur is used and are of no importance to the performance of the bur.

In order to study the effects of subsequent cuttings, 3 groups of burs of 6 dozen each were cut on a bur machine which had been given to the laboratory, on a loan basis, by a commercial bur manufacturer. These burs were sent to the manufacturer and received the commercial heat treatment. The flutes of the first group were cut twice; the second group, 4 times; and the third group, 6 times. The flutes of the burs in the first group had some fins present, while the second and third groups were composed of well-formed burs when they were examined under low-power magnification.

These burs were tested by cutting ivorene and brass under 250 and 500 gram loads with a length of engagement equal to 0.035 inch. Eighteen burs were used for each test run; 2 at each of 9 speeds between 1,000 and 12,000 r.p.m. The blocks of test material were weighed before and after each test on a chemical balance and the weight loss was calculated. These data were plotted as a function of the speed of rotation and the best graphical relationship was determined by the mathematical "method of least squares." From this relationship, the diameter of the bur, and the density of the material, the average linear displacement of the bur per flute revolution was calculated. The results are presented in the form of graphs, which show the average linear displacement per flute revolution, plotted as a function of the peripheral velocity. The results are shown in Fig. 1 for ivorene tests. This quantity, the

average linear displacement per flute revolution, represents the average amount that the axis of the bur moves along the cut per revolution of each flute. If the flute were to remove the material in a single chip, this quantity would be the thickness of the chip removed by each flute during each revolu-

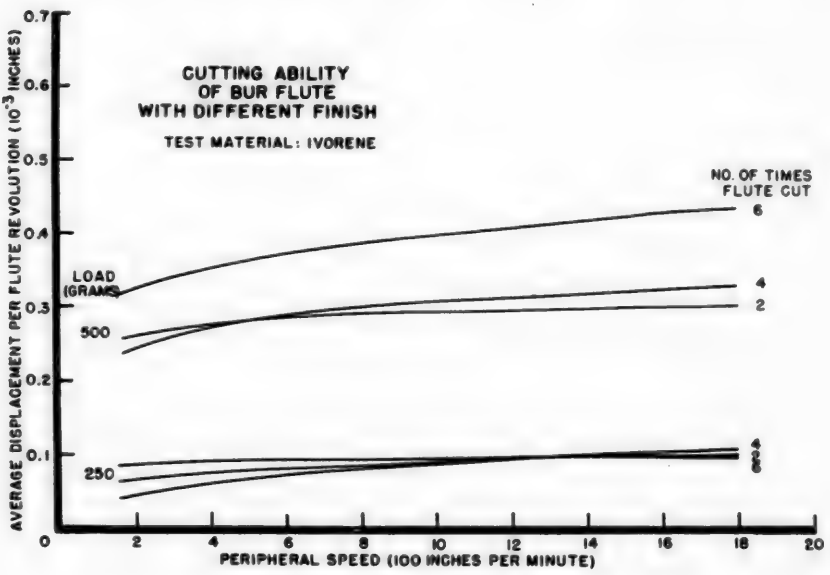


Fig. 1.

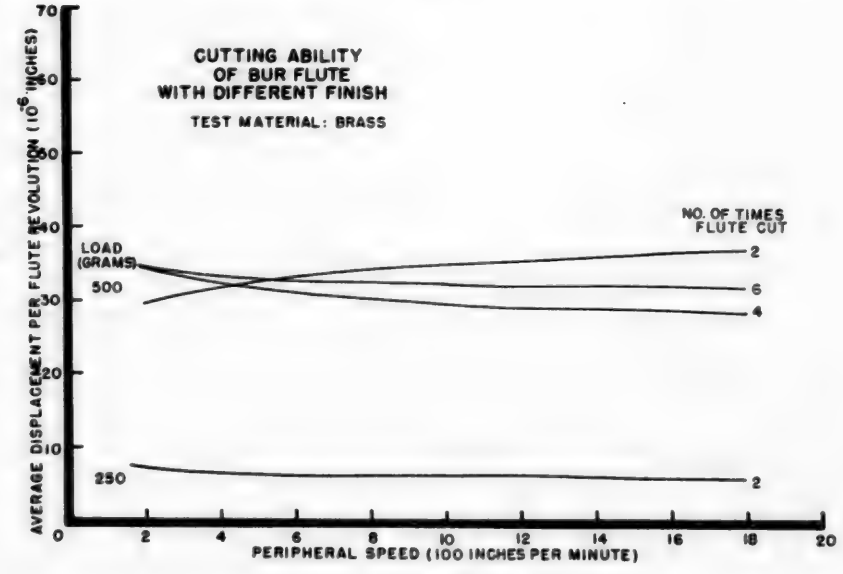


Fig. 2.

tion. When this quantity is plotted against the peripheral speed of the bur, the result is a very good representation of the cutting ability of the flute.

It is shown in Fig. 1 that at a 250 gram load, there is little difference between the performance of any of the burs. At a 500 gram load, the bur flute which was cut 6 times was superior to the flute cut twice and 4 times, the flute cut 4 times being very slightly superior to the flute that was cut twice.

The same relationship is shown in Fig. 2 when the burs were used to cut brass with a load of 500 grams. It was found that for these cuts the bur cut twice is the best cutter, the bur cut 6 times, the medium cutter, and the bur cut 4 times, the poorest cutter. The over-all differences between the 3 are very small and vary from zero to 25 per cent.

The most significant information offered in these figures is the order of magnitude of the average displacement per flute revolution. When cutting ivorene with a load of 500 grams this quantity is approximately 300 to 400 micro-inches (millionths of an inch). If the load is decreased to 250 grams, the quantity is approximately equal to 100 micro-inches. When the hardness of the material is increased, for example, brass, the quantity is approximately 35 micro-inches when testing with a 500 gram load and approximately 10 micro-inches when testing at a 250 gram load. These results correlate qualitatively with the N.B.S. films where it is shown that the cut material comes from the bur in the form of a dust cloud.

These graphs show very effectively the complex problem faced by bur manufacturers for it is evident that it is within the outer one-thousandth of an inch that the cutting properties of the flute are effective. Thus, any irregularties along the flute edge that are evident to the eye must show up as irregularities on the cut surface. If any excess fins are present, the flute proper never reaches the material, and if they break off from the flute, the cutting properties of the remaining bur flute determine whether or not the bur is a good cutter.

The results indicate that on a performance basis alone subsequent recuttings of the bur flutes form a better bur macroscopically but there is little difference at a few micro-inches from the very edge of the flute. Manufacturers say that most of the production cost occurs in the cutting process, and, if a bur is recut a subsequent number of times, the production cost increases directly without a corresponding increase in cutting ability.

THE EFFECT OF HEAT TREATMENT

Heat treatment is used to harden the bur that has been formed in soft steel. This operation must perform 2 tasks: (1) it must preserve the edge placed on the bur flute by the cutter and (2) it must harden the bur to increase its cutting life. Even though the cutter places a sharp edge on each flute, if the heat treatment process rounds that edge and destroys the sharpness, the bur will cut poorly.

Eighteen burs were formed on the bur machine and samples were photographed before and after heat treatment. The burs were also tested by cutting

ivorene with a 250 gram load before and after the heat treatment process. It was felt that testing in a soft material with light load would not destroy the edge placed on the bur flute before heat treating.

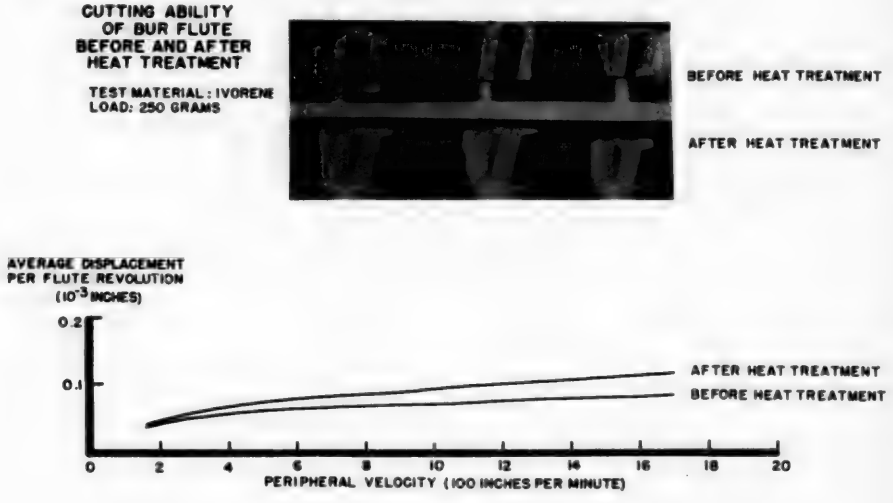


Fig. 3.

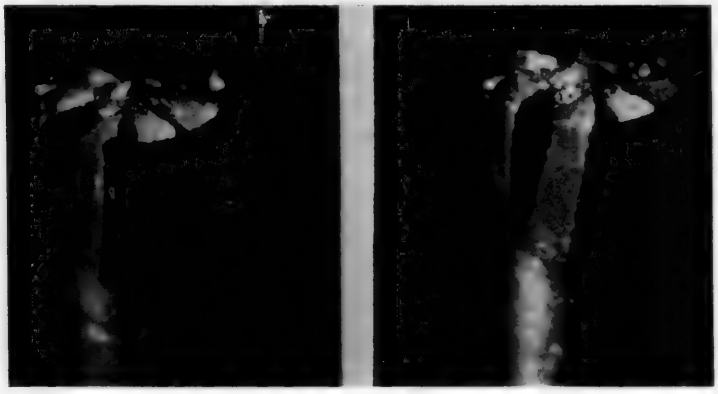


Fig. 4.

The results are shown in Fig. 3. The top half of the figure shows the photographic comparison and the bottom half shows the comparison of cutting results. The photographs show that, for the burs photographed, the flutes before heat treatment do not contain as many small irregularities as the same flutes after heat treatment. However, the cutting results indicate that the burs are as effective after, as they were before the heat treatment, for these test conditions.

THE EFFECT OF END FLUTES

Dental burs are formed with 2 different styles of end flutes: (1) the "Revelation" cut and (2) the "Star" cut. The end flutes of a "Star" cut head come together in a common junction at the axis of the bur, while the flutes of the "Revelation" cut bur come together at two junctions near a diametral cutting edge. This arrangement eliminates the noncutting center junction. For experimental purposes, burs may be formed without end flutes also. This entire study has been conducted with peripheral cutting and it is felt that the end flutes are inoperative.

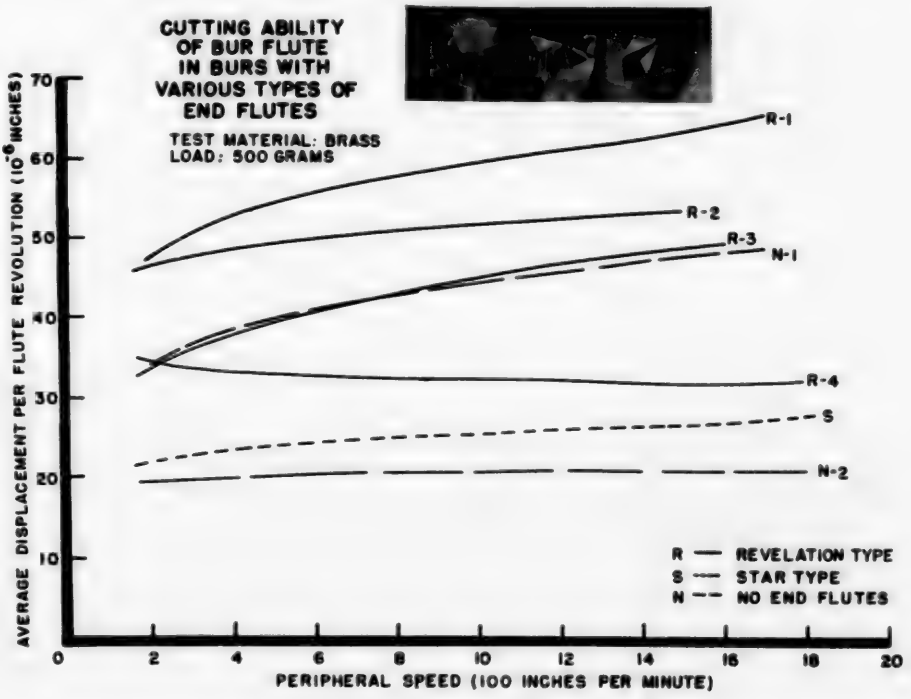


Fig. 5.

It is sometimes found when cutting brass that a "bearing" is formed at the end of the rotational axis of the bur, as shown in Fig. 4. This condition is more prevalent when testing burs with smaller diameters at the higher rotational speeds. Needless to say, the results should be questioned.

All test results, obtained from cutting brass with a 500 gram load by eight-fluted burs, the flutes of which were cut six times, are shown in Fig. 5. The results indicate that the "Revelation" type bur shows some superiority; however, there is considerable discrepancy between all curves, indicating that the ability to form equally effective side flutes on all burs is more important than the type of end flute utilized.

Emphasis must again be made that the tests were made by cutting peripherally. Plunge or oblique cutting tests, where the end flutes are directly involved, may not produce the same results. This should be the object of another study.

THE EFFECT OF CHANGING BUR DIAMETER

General theory indicates that the forces on each bur flute, when determined from the external load, do not depend on the diameter of the bur, but rather on the number of flutes and their rotational position. Thus, the average linear displacement per revolution and the length of cut under identical test conditions do not depend on the diameter of the bur. It follows that, because length of cut is constant, the volume of material removed will vary directly with the bur diameter as will the torque and the amount of mechanical energy that the engine is required to supply.

This same reasoning is substantiated experimentally. The curves of Figs. 1, 2, 3, and 5 show that the average displacement per flute revolution is nearly constant and does not depend to a great degree on the peripheral velocity. This quantity does increase slightly as the peripheral velocity is increased. This increase is felt to be due to increasing inertia forces, for in the test instrument the specimen is held in a rather heavy car while the bur is attached directly to the armature of a dental engine.

THE EFFECT OF CHANGING THE LENGTH OF ENGAGEMENT (DEPTH OF CUTTING)

Theory indicates that the external load (250 and 500 grams for these tests) is divided among the bur flutes that are actively cutting, resulting in certain forces on each flute. As the length of engagement is decreased, the force intensity on each small portion of the flute still cutting is correspondingly increased and the average displacement per flute revolution should also increase. This increase is so great that the volume of material removed by a shallow cut often exceeds that of a deeper cut.

Burs of standard design, that were formed in the laboratory, were tested by cutting brass with a 500 gram external load at cutting depths of 0.020, 0.030, and 0.040 inch. The results, given in Fig. 6, again show the average displacement per flute revolution as a function of the peripheral velocity. The results agree with theoretical considerations. For example, at a peripheral speed of 1,000 inches per minute (6,000 r.p.m.), it is found that for the particular group of burs tested, the average displacement was equal to 39 micro-inches when tested at a depth of 0.040; 60 micro-inches, at a depth of 0.030; and 110 micro-inches, at a depth of 0.020 inch.

THE EFFECT OF CHANGING THE NUMBER OF BLADES

As mentioned previously, the external load is distributed among the blades actively cutting. As the number of blades is decreased, the magnitude of the forces at each blade increases and the thickness of chip removed by each flute should correspondingly increase. It is interesting that, under cer-

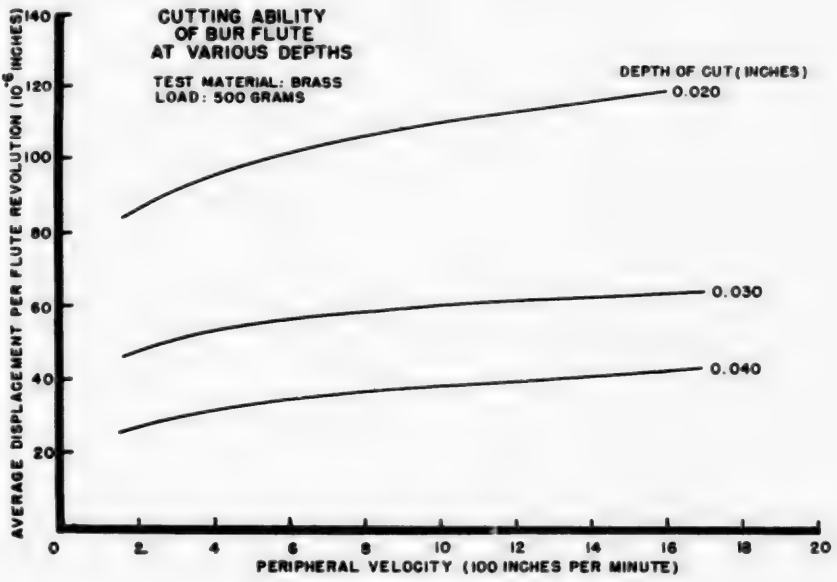

CUTTING ABILITY
OF BUR FLUTE
AT VARIOUS DEPTHS
TEST MATERIAL: BRASS
LOAD: 500 GRAMS
DEPTH OF CUT (INCHES)
0.020
0.030
0.040
AVERAGE DISPLACEMENT PER FLUTE REVOLUTION (10⁻⁶ INCHES)
PERIPHERAL VELOCITY (100 INCHES PER MINUTE)

Fig. 6.

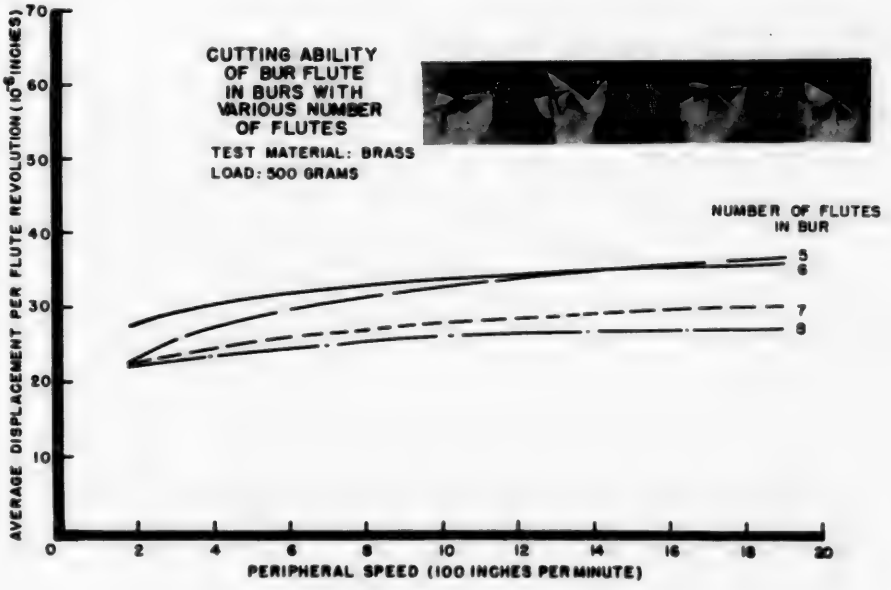

CUTTING ABILITY
OF BUR FLUTE
IN BURS WITH
VARIOUS NUMBER
OF FLUTES
TEST MATERIAL: BRASS
LOAD: 500 GRAMS
NUMBER OF FLUTES
IN BUR
5
6
7
8
AVERAGE DISPLACEMENT PER FLUTE REVOLUTION (10⁻⁶ INCHES)
PERIPHERAL SPEED (100 INCHES PER MINUTE)

Fig. 7.

tain conditions, theory indicates that nearly the same amount of material can be removed by either an 8-, 7-, 6-, or 5-fluted bur; i.e., the product of the chip thickness removed by each flute and the number of flutes may be nearly a constant. The reason for constructing burs with a smaller number of flutes arises from the increased space between the flutes which decreases the clogging tendency. Inasmuch as each flute is removing more material, the tendency for flute wear should be greater and the cutting life should be decreased.

It has been shown that in the fissure burs a bur with straight flutes produces less temperature rise than one with spiral flutes.[7] It has been reasoned that this may be due to the formation of a larger chip by the straight-fluted bur. The chip then carries some heat energy with it. Similar reasoning indicates that a bur with less flutes would be cooler operating. Again, there is a need for further study. A bur with a smaller number of flutes has also been shown to be more vibratory.

Four groups of 6 dozen burs each were formed and hardened. The first group was composed of burs with 8 flutes; the second group, 7 flutes; the third group, 6 flutes; and the fourth group, 5 flutes. These burs were tested and the results obtained when cutting brass with a load of 500 grams are shown in Fig. 7. In general, the results agree with theory. The 5-fluted bur was not as much superior to the 6-fluted bur as would be expected; in fact, at the lower values of peripheral velocity, the 5-fluted burs tested are inferior to the 6-fluted burs tested. This situation further confirms the supposition that it is very difficult to produce bur flutes of identical sharpness consistently.

SUMMARY

This report has presented briefly some results obtained during a testing program designed to show the effects of different design parameters on the performance of the inverted cone bur. These parameters are (1) flute finish, (2) heat treatment, (3) end flutes, (4) diameter of bur, (5) length of engagement or cutting depth, and (6) the number of flutes in the bur. The results have been expressed as graphs showing the average linear displacement per flute revolution as a function of the peripheral velocity. The average linear displacement per flute revolution is equivalent to the average distance that the bur advances into the cut per flute revolution. It has been found that this manner of data presentation demonstrates changes in edge-cutting ability very effectively.

It has been found that only the last one-thousandth of an inch of flute edge is effective for cutting a soft material. This distance decreases as the material hardness increases until only a few micro-inches of blade edge are effective in some cases. The largest manufacturing difficulty lies in producing equally sharp edges consistently; i.e., bur flutes that are identical within the outer one-thousandth of an inch.

The flutes of commercial burs usually have some fins or excess material. It may be removed by subsequent cuttings of the bur flutes; however, it has

been found that the small increase in the effectiveness of the bur does not warrant the extra cost. Heat treatment must retain the sharp edge placed on the flute by the cutter as well as harden the bur and increase its cutting life.

During peripheral cutting it is felt that the end flutes are inoperative. Test results indicate that the "Revelation" type flute is superior to the "Star" type end flute and the bur without end flutes. However, the results are not conclusive.

It has been found experimentally, as well as theoretically, that the average linear displacement per flute revolution does not depend markedly on velocity, indicating that the length of cut is nearly constant irrespective of reasonable changes in bur diameter. It is assumed in this statement that the edge conditions at the flutes as well as the test conditions are the same.

Changing the length of engagement as well as the number of flutes changes the manner in which the external load is distributed among the flutes of the burs. If the force on the flute is increased, the average displacement per flute revolution is increased. This occurs when either the length of engagement or the number of flutes is decreased.

REFERENCES

1. Henry, E. E., and Peyton, F. A.: A Study of the Cutting Efficiency of Dental Burs for the Straight Handpiece, *J. D. Res.* **30**: 854-869, 1951.
2. Henry, E. E., and Peyton, F. A.: The Relationship Between Design and Cutting Efficiency of Dental Burs, *J. D. Res.* **33**: 281-292, 1954.
3. Lammie, G. A.: Critical Review of Cutting Instruments in Cavity Preparation. 2. The Tungsten Carbide Bur, *Internat. D. J.* **4**: 43-53, 1953.
4. Staunt, M.: Personal communication.
5. High Speed Motion Picture Study of Dental Burs, *J. Dist. Columbia D. Soc.* **29**: 9-10, 1954.
6. Bryton, B., Skinner, E. W., Lindenmeyer, R. S., and Lasater, R. L.: The Cutting Effectiveness of a Dental Bur as Related to Its Design, *J. D. Res.* **33**: 693, 1954.
7. Vaughn, R. C., and Peyton, F. A.: The Influence of Rotational Speed on Temperature Rise During Cavity Preparation, *J. D. Res.* **30**: 737-744, 1951.

HISTOGENESIS AND HISTOCHEMISTRY OF PULPAL CALCIFICATION

PERCY L. JOHNSON AND G. BEVELANDER

Department of Histology, Murray and Leonie Guggenheim Institute for Dental Research, and the College of Dentistry, Graduate School of Arts and Science, New York University, New York, N. Y.

CALCIFICATION in the pulp of human teeth occurs in the form of pulp stones, and as areas of diffuse calcifications in a relatively restricted area. Calcified structures have long been recognized and were described and commented upon by Bodecker,[2] Salter,[10] Black,[1] Preiswerk,[9] and others.

The origin of denticles has been ascribed to several different causes. Fridrichovsky[4] maintained that denticles develop from a folding of embryonic tissue which normally would form dentin. Orban[7] asserted, on the other hand, that epithelial rests trapped in the pulp tissue initiate cellular activity resulting in the formation of denticles. Hill[5] expressed the opinion that the formation of denticles is associated with certain stellate cells of the pulp which function in building an irregular dentin around a calcific deposit.

It was formerly held that the occurrence of pulpal calcifications was related to irritation, trauma, caries, etc. They were also causally associated with neuralgia and referred pain of various kinds. The current concept in regard to the origin and presence of pulpal calcifications, according to Thoma,[11] Boyle,[3] and Hill,[5] differs from the ideas referred to above in two important respects: (1) a differentiation is made between the occurrence of pulpal calcifications resulting in denticle formation and areas of the pulp considered pathologic (necrotic) which subsequently undergo calcification; and (2) referred pain is no longer ascribed to the presence of pulpal calcifications.

It is the purpose of this investigation to record our observations on the occurrence and structure of calcifications in the pulp. In addition, we shall describe, in so far as possible, the histogenesis and histochemical components of these structures. Finally, we shall compare our observations on pulpal calcifications with the histogenesis of bones and teeth.

MATERIALS AND METHODS

Human teeth obtained immediately after extraction were used in this investigation. Following extraction the teeth were bisected transversely or longitudinally and placed at once in fixing fluid. Some of these specimens were then infiltrated with paraffin prior to the preparation of thin slabs cut by means of a copper disk impregnated with diamond dust. Still other teeth were cracked in a

This investigation was supported (in part) by a research grant from the National Institute for Dental Research of the National Institutes of Health, United States Public Health Service, Bethesda, Maryland.

Received for publication April 15, 1955.

vice, fixed and the pulps removed intact, sectioned and treated as desired. Finally, in another group of teeth, the apical end of the tooth was cut off, the coronal portion then placed in the fixer, decalcified, and subsequently embedded in paraffin or celloidin. The fixing fluids used were chosen to reveal the several histochemical components identified as follows: Helly's fluid followed by 0.05 per cent toluidine blue for metachromasia, 80 per cent alcohol for the Gomori phosphatase test and the Von Kossa test for mineral deposits; Bouin's fluid for hematoxylin and eosin preparations, Carnoy's fluid for the McManus-Schiff reaction for glycoprotein, glycogen and nucleic acid demonstration. Comparison of sections prepared by means of the cutting wheel with those cut with the microtome showed relatively little distortion.

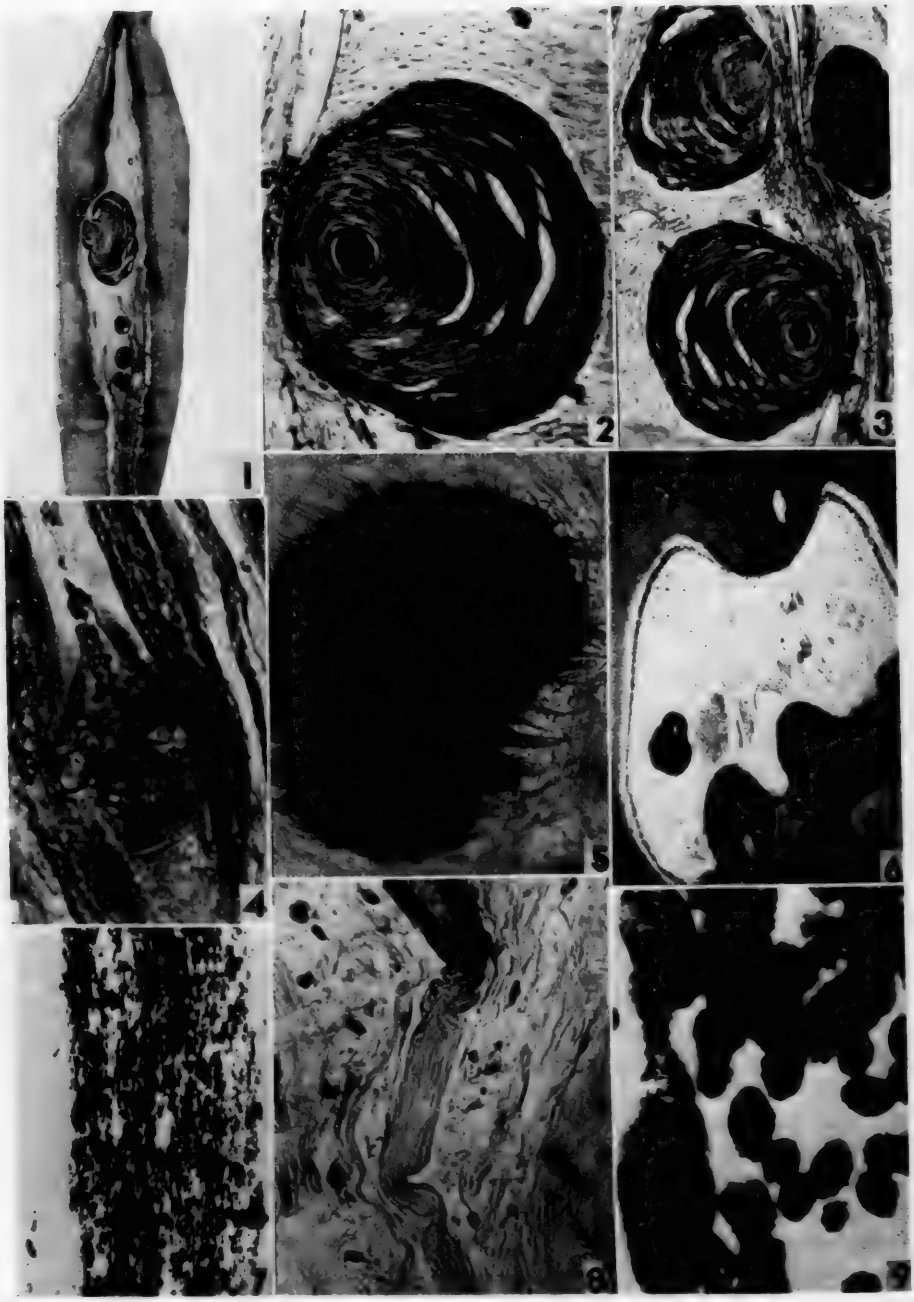
OBSERVATIONS

A. *Pulp Stones.*—The numbers and sizes of pulp stones in the pulp of human teeth were observed to vary greatly (Fig. 1). While some teeth are devoid of pulp stones, others were observed to contain from 1 to 12 or more. The size of these stones was sometimes so small as to be barely perceptible, while others consisted of large conglomerate fused masses occupying the greater part of the pulp cavity (Fig. 18). Pulp stones, according to our observations, usually occur in the pulp horns and only occasionally are they observed in the region of the root canals.

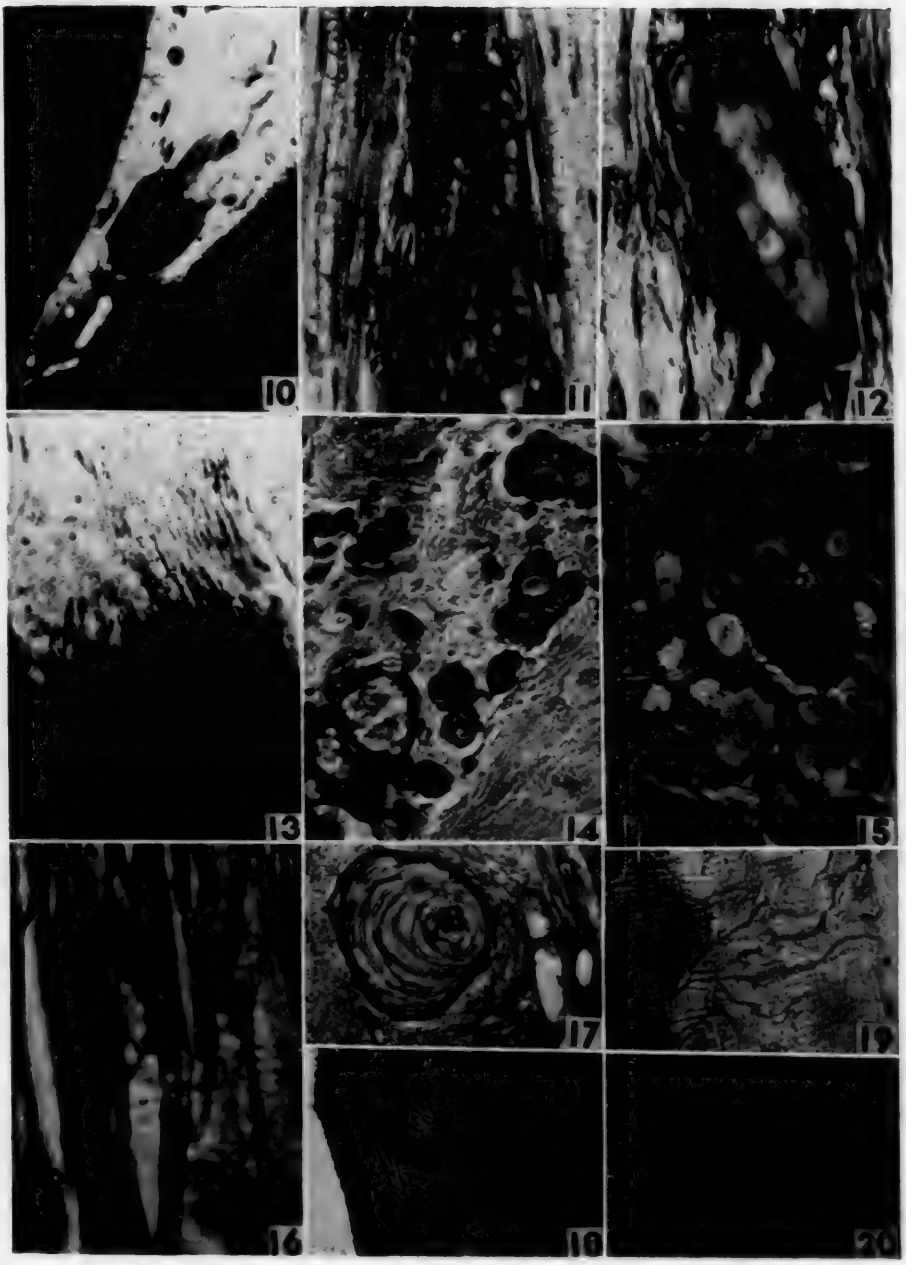
Pulp stones in the fresh state appear quite uniformly transparent in contrast to dentin and enamel. When the pulp was examined with the aid of a binocular dissecting microscope it was sometimes necessary to probe the pulp stones in order to ascertain that they were actually calcified bodies and not large vacuoles filled with transparent fluid. When subjected to decalcification with acids, they behaved like dentin and enamel in that they did not effervesce.

During the course of this investigation we have devoted considerable attention to the manner in which pulp stones arise. In so far as we have been able to ascertain, the precursors upon or around which these structures arise are cell nests (Figs. 2 and 4). Subsequently, these cells become enclosed by concentrically arranged fibers; later this complex is impregnated with mineral salts. The pulp stone is elaborated in successive stages. Between each calcified increment there is a prominent fibrous junction. The periphery of the structure is surrounded by a thick zone of uncalcified fibers which we have ascertained to be reticular connective tissue. The fibers which are incorporated in the pulp stones are derived from the surrounding connective tissue (Fig. 5). A variation of this situation is shown in Fig. 8, which illustrates a calcified mass embedded in a condensation of collagenous fibers.

More complex arrangements of pulp stones are also of frequent occurrence. The complexity consists of increased size and fusion of one or more of the bodies. In the example just cited the fusion is effected by a fibrous union (Fig. 10).



Figs. 1 to 9.—Fig. 1. Denticles of decalcified tooth (H & E, orig. mag. ×10). Fig. 2. Denticle (McManus-Schiff, orig. mag. ×300). Fig. 3. Denticles impinging on nerve bundle (McManus-Schiff, orig. mag. ×150). Fig. 4. Beginning formation of denticle (Eosin-Azure, orig. mag. ×750). Fig. 5. Matted fibers surrounding developing denticle (H & E, orig. mag. ×200). Fig. 6. Free, embedded and adherent denticles (H & E, orig. mag. ×80). Fig. 7. Diffuse calcifications in pulp (Von Kossa, Light green, orig. mag. ×50). Fig. 8. Collagenous fibers approaching and surrounding denticle (H & E, orig. mag. ×750). Fig. 9. Diffuse calcifications in pulp (Von Kossa, orig. mag. ×1,200).



Figs. 10 to 20.—Fig. 10. Fusion of two denticles with fibrous matrix (H & E, orig. mag. ×50). Fig. 11. Early stages in diffuse calcification between nerves (Metachromasia, T. Blue, orig. mag. ×900). Fig. 12. Enlarged portion of diffuse calcification (Metachromasia, T. Blue, orig. mag. ×1,200). Fig. 13. Fibrous component of denticle with Von Kossa positive granules oriented along fibers (orig. mag. ×1,200). Fig. 14. Early formation of diffuse calcifications (Schiff, orig. mag. ×900). Fig. 15. Early formation of diffuse calcifications (Foote stain, orig. mag. ×900). Fig. 16. Myelinated nerve fiber with node of Ranvier (M. Blue, orig. mag. ×1,200). Fig. 17. Denticle (Metachromasia, T. Blue, orig. mag. ×100). Fig. 18. Denticles adherent to one another (H & E, orig. mag. ×400). Fig. 19. Embedded fibers in body of denticle (orig. mag. ×1,200). Fig. 20. Enlarged denticle with dentinal tubules about periphery (H & E, orig. mag. ×900).

In those pulp stones which arise or lie in proximity to dentin, the presence of odontoblasts on the peripheral aspect of these bodies was frequent. Subsequently, a calcified tissue resembling dentin appears to be deposited on the periphery of the pulp stones. Such a denticle, at first free, may later become adherent and subsequently become incorporated into the dentinal tissue appearing as an integral part of the calcified dentin (Fig. 6). It is possible, as indicated above, to trace the origin and development of the incorporated denticle; the original free pulp stone can be identified as an area unlike dentin in appearance surrounded by dentinal tubules which take a different direction from the regularly arranged dentin surrounding this mass (Fig. 20).

Structures such as blood vessels and nerves do not, in our opinion, contribute or serve to initiate the formation of pulpal calcifications. Fig. 3, for example, illustrates a typical relationship between a group of nerve fibers and pulp stones. The fact that the pulp stones are in such intimate relation to the nerve that they encroach upon it appears to be incidental and does not implicate the nerve as a nidus for this type of pulpal calcification (Fig. 16).

B. *Diffuse Calcifications.*—Calcifications of this variety occur as unorganized masses in the apical part of the root canal (Fig. 7). They appear as multiple accretion centers separated from one another by the ground substance of the pulp. As enlargement occurs, they elongate in the direction of the long axis of the pulp. These accretions upon superficial examination appear amorphous. Examination under higher magnification, however, reveals that they consist of minute spheres or ellipsoids (Fig. 9). The ellipsoidal character of these bodies is the result of the fusion of neighboring accretions (Figs. 11 and 12). The process of fusion continues and eventually results in the formation of fluted columns of calcified cylinders attached at their sides and united by calcified material devoid of a fibrous matrix. This process results in extensive calcification of the root canal. These masses may impinge upon vessels and nerves which may in turn cause changes in structure and function. Despite the intimate association of these structures with vessels or nerves, we have found no evidence that they are in any way concerned with the origin or development of these calcified structures.

HISTOCHEMICAL OBSERVATIONS

A. *Phosphatase and Glycogen.*—Pulps of several adult teeth were examined for the presence of alkaline phosphatase by means of the Gomori method. Some of these pulps were normal, others showed retrogressive changes, and still others revealed definite pathosis. Phosphatase appeared in these pulps in one locus only, the peripheral part of the pulp cavity in the region of the odontoblasts. This observation augments our earlier studies in which we described the diminution of this enzyme in the pulp as the tooth matures. Of particular interest was the absence of phosphatase in any of the components making up or surrounding the pulpal calcifications.

Tests for the presence of glycogen using the McManus-Schiff reagent, Best's carmine and iodine methods were completely negative. Our previous

studies on embryonic teeth showed that glycogen disappears before calcification of the tooth begins. In comparing the formation of enamel and dentin with the formation of pulp stones, we find that glycogen is present before calcification, phosphatase before and during calcification of dentin and enamel, but, in the proximity of pulp stones, these substances are not present during any stage of their existence. This finding lends additional support to the concept that calcification can occur in the absence of both phosphatase and glycogen.

B. *Glycoprotein.*—The formation of pulp stones and areas of diffuse calcification takes place in an environment consisting of fibers and ground substance showing a pronounced glycoprotein staining reaction (Fig. 14). As calcification proceeds or after it has taken place, the fibers surrounding the pulp stone are intensely Schiff-positive, while the calcified area responds very slightly to this treatment, both with respect to the fibers and ground substance. Application of the Foote technic to pulp stones (Fig. 15) indicates that the peripheral fibers have the characteristics of reticular fibers. It is obvious that during calcification certain changes occur in the calcified mass which result in a marked diminution of the periodic-Schiff reaction.

C. *Nucleic Acids and Basophilia.*—In view of our previous observations[6] in which we observed a pronounced nucleic acid localization in odontoblasts and ameloblasts associated with the formation and calcification of dentin and enamel, respectively, we directed our attention to the nucleic acid content of the pulpal cells in adult teeth. Comparison of nucleic acid localization of both RNA and DNA in cells in close proximity to the pulp stones and various other cells in the pulp were essentially similar in respect to the presence of nucleic acids. The visualization of these substances was extremely weak in all of the pulp cells. In regard to nucleic acids we cannot, on the basis of our observations, implicate them in the formation or calcification of pulp stones.

D. *Von Kossa.*—Pulp stones in varying degrees of development treated according to the Von Kossa method result in a negative reaction during the early stages of development. Larger and more highly developed pulp stones show positive (black) central areas while the peripheral portion remains negative. Subsequent staining by safranin O or the McManus-Schiff reaction reveals that the periphery of these structures is fibrous and, as previously mentioned, when treated according to the Foote method they appear to be reticular fibers. The sequence of events in terms of calcification therefore is similar to that observed in other calcified tissues in that an organic phase precedes calcification.

Examination of areas of diffuse calcification tested by the methods mentioned in the previous paragraph shows a striking similarity. The youngest bodies reveal ground substance and reticular fibers, and the center shows the first sign of calcification; when calcification takes place, the fibers and ground substance react faintly, if at all, to the McManus-Schiff reaction.

One of the most interesting observations made on Von Kossa-treated material is shown in Fig. 13. This photomicrograph illustrates the peripheral fibers of a growing pulp stone. It further depicts the presence of minute beads of

Von Kossa positive material arranged in linear fashion along the fibers. These structures were examined with a phase contrast microscope and with the highest magnification available to us using the compound light microscope. From these observations we conclude that these mineral bodies are essentially an integral part of the fiber. Evidence for this conclusion is based upon our examination of this fiber complex which shows that internodes of organic material connect each bead of calcified material. The significance of this observation is, we believe, in the clear demonstration of the intimate association shown between fibers and calcifying material.

In diffuse areas of calcification we observed (Fig. 9) that isolated concretions of calcified globules reveal small buds extending over most of the peripheral surface. In these buds we observed no fibrous matrix, but a pronounced metachromatic ground substance in the peripheral area is, nevertheless, present.

Metachromasia.—Adult pulps treated with toluidine blue show a relatively weak or negative metachromatic response with the exceptions noted below. The region of predentin is markedly metachromatic. This is also true for those areas of the pulp which appear to be or are elaborating pulpal calcifications. In those areas in which diffuse calcifications are being formed the metachromatic ground substance extends for a considerable distance beyond the actual area undergoing calcification. In the areas where calcification has occurred, each individual center is surrounded by a marked metachromatic peripheral zone.

Metachromasia observed in pulp stone formation is in most respects similar to the situation described above. The uncalcified organic phase is intensely metachromatic; as calcification occurs the metachromasia is reduced or lost in the calcified matrix but is still prominent in the peripheral fibrous zone. Pulp stones made up of several increments show metachromasia at the interfaces of the increments and peripherally.

In the pulp calcifications we have studied, we observed that both glycoprotein and acid polysaccharides are components of the ground substance of those areas which subsequently calcify. The fact that the metachromatic substance was not removed by hyaluronidase digestion suggests that the acid polysaccharide may be chondroitin sulfate.

DISCUSSION

Pulpal calcifications, usually referred to as pulp stones, may be grouped, according to our observations, into two categories: denticles and areas of diffuse calcification. We observed the denticles to be located in the region of the pulp horns and the areas of diffuse calcification in the root canals. This is in agreement with the accepted views now generally held.

Denticles arise from a nidus of cells. Subsequently, reticular fibers surround the cells and then calcification of the initial organic matrix occurs at the same time a peripheral organic matrix is laid down. Extensive calcification of the pulp horns, sometimes observed, results from the fusion of several

individual denticles. Attached and imbedded denticles originate as free bodies. Their subsequent attachment or incorporation into dentin is the result of dentin formation on the free surface of these structures. The only denticles we observed which reveal dentinal tubules are the heterogeneous structures made up in part of concentric lamellae of calcified matrix—the free denticle, surrounded in whole or in part by dentin. We conclude from our observations that the differentiation between "true" and "false" denticles is untenable. Denticles are false, that is, they do not contain dental tubules. Subsequently, they may, in the process of attachment to, or incorporation by dentin, be surrounded by tissue containing dentinal tubules.

Areas of diffuse calcification were observed to be localized in the root canals. They were observed in early development as minute spheres which undergo subsequent enlargement due to the fusion of neighboring centers. Like the denticles, these structures also have an organic matrix composed of reticular fibers and a ground substance.

The histochemical observations made in reference to pulpal calcifications are, we believe, of more than routine interest when compared with similar studies conducted on growing calcifying tissues such as dentin and enamel. A precursor complex consisting of reticular tissue, and a ground substance containing mucoprotein and metachromatic components was observed for denticles and diffuse calcifications. This is also found in bone, dentin, and cementum. Osteoblasts, odontoblasts, and ameloblasts contain considerable amounts of phosphatase, glycoprotein, and nucleic acids during the time calcification takes place. It appears that pulp stones have an acid polysaccharide and a mucoprotein precursor in common with other regions of calcification. Unlike them, neither phosphatase nor (by implication) phosphorylase are components of the calcific structures which arise in the pulp.

Many factors relating to pulpal calcifications still remain in the realm of speculation or obscurity. It is not clear whether calcification in the pulp is correlated with retrogressive changes or pathosis, whether this phenomena is a protective mechanism or an exhibition of the multipotency of the pulp constituents. The present studies do serve, we believe, as a basis for comparison between events which happen in the pulp and in other regions which calcify, particularly those organs subject to aberrant forms of calcification.

CONCLUSIONS

1. A study of pulpal calcification in the human adult tooth has been made. According to our observations, there are basically two varieties of pulp stones: denticles consisting of a concentrically arranged lamellae and areas of diffuse calcification.

2. Denticles occur in the pulp horns. They are at first free but they may become attached or embedded. In this process they acquire tissue containing dentinal tubules. We find no indication that denticles are "true" or "false."

3. Areas of diffuse calcification are confined to the pulp canals. They appear first as minute centers which fuse with neighboring bodies, eventually giving rise to the columnar or cylindrical structures which have frequently been illustrated.

4. Histochemical tests to which the pulp was subjected reveal that reticular connective tissue fibers, mucoprotein, and acid polysaccharides were constant constituents of the loci subsequently giving rise to denticles or diffuse calcification. Nucleic acids, alkaline phosphatase, and glycogen commonly found in cells associated with the calcification of bones and teeth were not present.

5. The possible significance of pulpal calcifications is discussed and comparison is made between normal and aberrant calcifications.

REFERENCES

1. Black, G. V.: The American System of Dentistry, 1886, Vol. 1, pp. 860-880.
2. Bodecker, C. F.: Anatomy and Pathology of the Teeth, 1894.
3. Boyle, P. E.: Kronfeld's Histopathology of the Teeth, Philadelphia, 1950, Lea & Febiger.
4. Fridrichovsky, J.: Zur Histologie der Denticle, *Ztschr. f. Stomatol.* **25**: 124, 1927.
5. Hill, T. J.: Textbook of Oral Pathology, Philadelphia, 1949, Lea & Febiger.
6. Johnson, P. L., and Bevelander, G.: The Localization and Interrelation of Nucleic Acids and Alkaline Phosphatase in the Developing Tooth, *J. D. Res.* **33**: 128-135, 1954.
7. Orban, B.: Epithelial Rests in the Teeth and Their Supporting Structures, Proc. Am. Assoc. Dent. Schools, pp. 121-133, 1929.
8. *Idem:* Oral Histology and Embryology, St. Louis, 1953, The C. V. Mosby Company.
9. Preiswerk, G.: Lehrbuch und Atlas der Zahnheilkunde, Bd. 30, Munchen, 1908.
10. Salter, J. A.: Dental Pathology and Surgery, Baltimore, 1875, William Wood & Company.
11. Thoma, K.: Oral Pathology, St. Louis, 1941, The C. V. Mosby Company.

ACCOMMODATION OF NORMAL AND PATHOLOGIC PULP NERVES

FERNANDO VARGAS F.*

Instituto de Fisiología y Escuela Dental, Universidad de Concepción, Chile

AS early as 1849, Du Bois-Reymond had noted the increment of the threshold of an excitable tissue, when stimulated with electric currents of increasing rise-times, and formulated the following law: "Excitation is a function not only of unit intensity but also of its rate of development (dc/dt); the more rapid the change the greater the excitant effect." Later, Nernst[1] named this property of excitable tissues "accommodation."

The results of various experiments[2-4] suggest that stimulation with electric currents of long rise-times is a useful index of the velocity of reaction of the physical and chemical processes which tend to re-establish membrane equilibrium. Tissue with fast reactions will require more rapidly increasing currents to obtain stimulation than those with slower processes.

Since accommodation is probably a membrane phenomenon, it is to be expected that physical and chemical changes which alter the membrane's properties will be reflected by changes in the response to linearly or exponentially increasing stimuli. Thus, lack of Ca^{++}, K^+, CO_2 and alkalosis have been shown to depress accommodation, as do also drying, cold, or trauma. An excess of Ca^{++}, K^+, acidosis, and ischemia increases accommodation.[5-15]

The variations of accommodation, produced by changes of the excitable tissue, make the use of linearly or exponentially increasing currents a useful method of stimulation for the study of pathologic processes. In man, for instance, neuromuscular accommodation has been studied in relation to changes in calcium metabolism, wallerian degeneration, paralysis, and alkalosis.[16-18] Accommodation of teeth in relation to pulpitis has also been investigated.[19]

We consider that the study of accommodation of nerve fibers of the dental pulp is of great theoretical and practical value, especially in relation to the behavior of this tissue in comparison to other sensitive nerves, and as a possible tool for the diagnosis of pathologic changes in the pulp.

MATERIAL AND METHODS

For this study 56 patients, attending the clinics of the Dental School of the University of Concepción, were used. In this group there were 16 men and 40 women, between 20 and 40 years of age.

The accommodation of normal and abnormal teeth was studied. Caries-free teeth with normal periodontium were considered as controls, and teeth with clear-cut symptoms of pulpal inflammation, usually secondary to deep

Received for publication March 2, 1955.

*Present address: Department of Zoology, University of Chile, Santiago, Chile.

caries, were chosen as pathologic. Only those cases which complied with the following conditions were utilized for the present study: (1) constant electric resistance of the teeth throughout measurement, (2) no variation of rheobase greater than 10 per cent, and (3) measurement of threshold with 8 currents of different time-constants.

A case history was made of each patient, in which clinical symptoms and diagnosis, general state of the mouth, decayed teeth, etc., were noted, together with the results of the electrophysiologic measurements.

Exponentially increasing electric currents, instead of those rising linearly, were used because the latter are technically more difficult and expensive to produce.

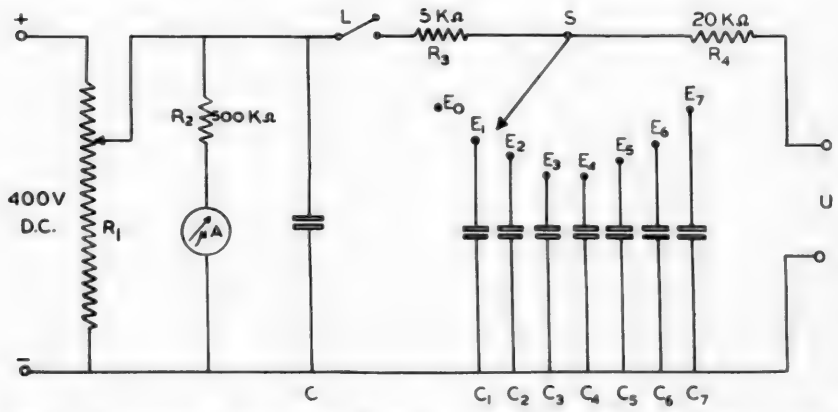


Fig. 1.—Schematic representation of the stimulating apparatus. The potentiometer R_1 regulates D.C. voltages, which are measured by the instrument μA and stabilized by condenser C of high capacity. Switch L closes the circuit, and selector S is used to obtain the different time-constants by connecting the condensor C_1 to C_7 respectively. When S is in position E_0, a square wave is obtained. The stimulating electrodes are connected to U.

Apparatus.—The instrument used to produce the exponential currents consists of: (1) D.C. power supply with voltage control, (2) a voltmeter which indicated the voltage of the stimulating current, and (3) a system of condensors in parallel, which are connected as required to the circuit to obtain a predetermined time-constant (Fig. 1).

This instrument produces a square wave impulse and seven exponentially increasing currents of different time-constants* (Table I), depending on the values of the variable resistance and of the condensors connected to the circuit.

The characteristics of the stimulating currents were studied,[2] using a Cossor double-beam cathode ray oscilloscope and a Grass photographic camera. The square wave current is referred to as E_0 and the exponentially increasing currents as E_1, E_2, E_3, E_4, E_5, E_6 and E_7 (Fig. 2).

Measurement of Ohm Resistance.—A Wheatstone bridge (Heathkit) was used to measure the resistance of the teeth before and after each series of

*Time-constant equals resistance multiplied by capacity (RC).

threshold determinations. The electrode used in our studies is of simple design, easy to place, remains fixed, and has low resistance. The active pole consists of a thin insulated wire whose metallic end is fixed to the tooth by Plasticine. The indifferent pole is a metal plate which is fixed on the forearm. Both electrodes are covered with conducting paste.

We have also used another type of electrode of constant contact surface to study initial and final resistance. This electrode was formed by a hollow-rubber cup stuck to the tooth and filled with conducting paste.

Measurement Technic.—The subject sits in a dental chair and is instructed about the study to which he is to be submitted, the painlessness of the procedure being especially stressed. The preliminary conditioning of the patient is of great importance, for the response to the electric stimuli depends on the emotional state of the subject under study.

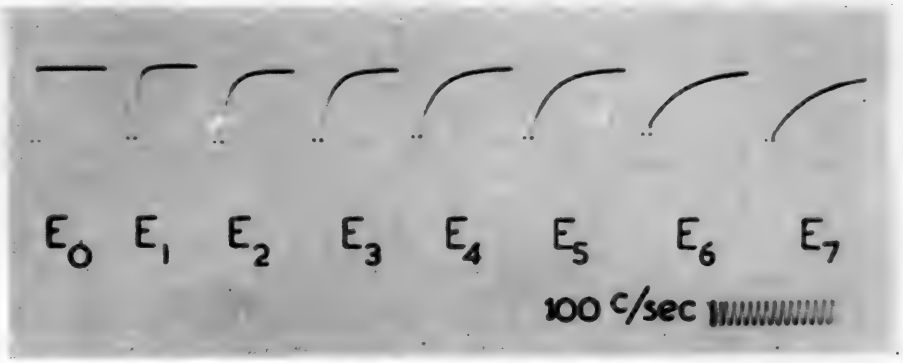


Fig. 2.—Cathode-ray oscilloscope tracings of the different stimulating currents obtained with the same voltage. Frequency of the time signal is 100 c/sec.

The tooth to be examined is carefully isolated by means of cotton, and the surface dried; the dental electrode is then attached by means of a drop of Plasticine, previously melted by heat; subsequently, conducting paste is placed on the metal point in contact with the tooth.

TABLE I
TIME-CONSTANTS OF THE EXPONENTIAL INCREASING CURRENTS IN MILLISECONDS (MS)

CURRENT	E_1	E_2	E_3	E_4	E_5	E_6	E_7
Time-constant (ms)	4.85	9.75	12.25	18.75	21.25	37.50	42.50

These preliminary steps over, the electric resistance of the tooth is measured several times until a constant value is obtained; this usually requires several minutes. After this, stimulation with a square wave current is commenced, and the rheobase established; next, stimulation is continued with exponentially increasing currents of progressively longer time-constants. The weakest sensation which stimulation elicits in the patient, and which he indicates by a movement of his hand, is used to determine the threshold. Suffi-

cient time is allowed to elapse between each stimulation in order to avoid the effects of polarization which are prone to occur with currents of the longest time-constant. At the end of the series of measurements, rheobase is again determined with a square wave impulse.

RESULTS

Ohm Resistance of Teeth.—The values of resistances found, using the constant surface electrode (11.5 mm.²), are approximately the same as those obtained by Wertz (cit. Björn),[20] who used an electrode with a 12 mm.² surface.

On the average, resistance fell to about 53 per cent of the initial value, and became stabilized at this level. It is of great importance that any neurophysiologic determination should be made only after the electric resistance of the tooth has become constant, otherwise gross errors will be committed.

Table II presents a summary of the resistance measurements. R_1 refers to the initial and R_2 to final resistance. The mean value of the former was 0.7 megomhs (MΩ) and of the latter 0.29 MΩ. This difference is statistically[21] significant ($P < 0.001$). In 2 cases no change in resistance was observed.

TABLE II
RESISTANCE CHANGES OF TEETH

CASE NO.	TOOTH NO.	INITIAL RESISTANCE R_1 (MΩ)	FINAL RESISTANCE R_2 (MΩ)	PER CENT OF RESISTANCE DECREASE
5	9	1.000	0.650	35
6	6	1.000	0.520	48
9	7	0.009	0.005	39
10	23	0.050	0.005	90.2
17	8	0.040	0.008	78.8
20	11	1.500	0.018	98.8
28	6	2.200	0.580	72.7
29	9	0.700	0.340	51.5
32	22	1.400	0.020	98.6
33	7	0.600	0.082	86.4
36	22	1.000	0.350	65
37	6	0.150	0.150	0
42	13	0.175	0.064	63.5
45	20	0.120	0.100	16.7
47	22	0.700	0.500	28.6
50	11	0.100	0.021	79
51	11	1.400	0.450	68
52	8	1.000	1.000	0
53	9	0.400	0.350	12.5
54	6	0.800	0.500	37.5
55	8	1.000	0.670	33
62	6	1.100	0.090	91.9
63	7	0.560	0.400	29
66	6	0.320	0.130	59.4
TOTAL = 24 CASES		MEAN VALUE $M_1 = 0.705$	MEAN VALUE $M_2 = 0.297$	MEAN VALUE $M = 53.46 \pm 6.2$*

*The arithmetic mean ± the standard error (SE) is indicated.

Accommodation of Pulp Nerves.—Of the 56 subjects in which accommodation was measured, only 35 complied with the pre-established conditions, 20 of these being normal and 15 pathologic.

A graph relating threshold to time-constant was drawn for every subject. All threshold values were expressed as multiples of rheobase: V_1/V_0;

V_2/V_o; V_3/V_o, etc., where V_1, V_2, V_3 refer to the threshold for the corresponding exponential currents E_1, E_2, E_3, while V_o represents rheobase, the threshold voltage for a square wave impulse. The mean value of the results of normal and pathologic cases was also calculated in order to obtain representative curves. The slope of the curves was measured by determining the tangent of the angle formed by the initial part of the curve and the abscissa. This slope was expressed in rheobase per millisecond (Fig. 3).

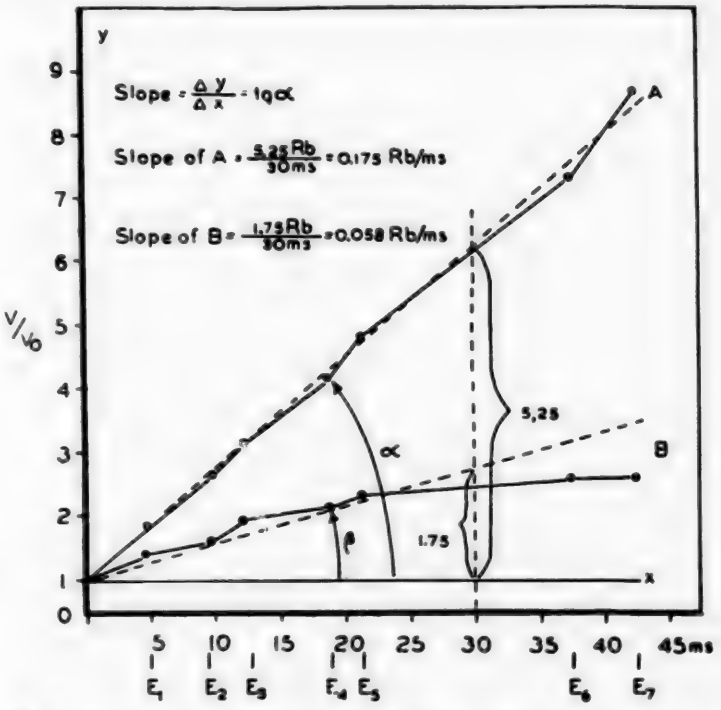


Fig. 3.—Graphic representation of the threshold ratios (ordinate) as a function of the different time-constants of the stimulating currents (abscissa). V_o represents the rheobase and V the threshold of the exponentially increasing currents. Curve *A* corresponds to a normal tooth; curve *B* to a pathologic one.

Normal Teeth.—The accommodation of normal teeth is high; this is shown by the fact that the threshold for a current with a time constant of 42.5 ms is 7.75 rheobase (rb). The slope of the accommodation curve in the normals (Table III) is 0.164 rb/ms. As shown in Fig. 4, threshold increases linearly as a function of the time-constants of the exponential currents.

Experimentally it has been established that accommodation suffers a "breakdown,"[5, 8, 22] that is, a clear-cut decrease of slope appears after a certain value of the time-constant of the stimulating current. In other words, once a certain value of time-constant is reached, the threshold does not continue to rise with the same velocity as initially. In the case of normal dental pulp nerves, "breakdown" generally does not occur until stimulation with

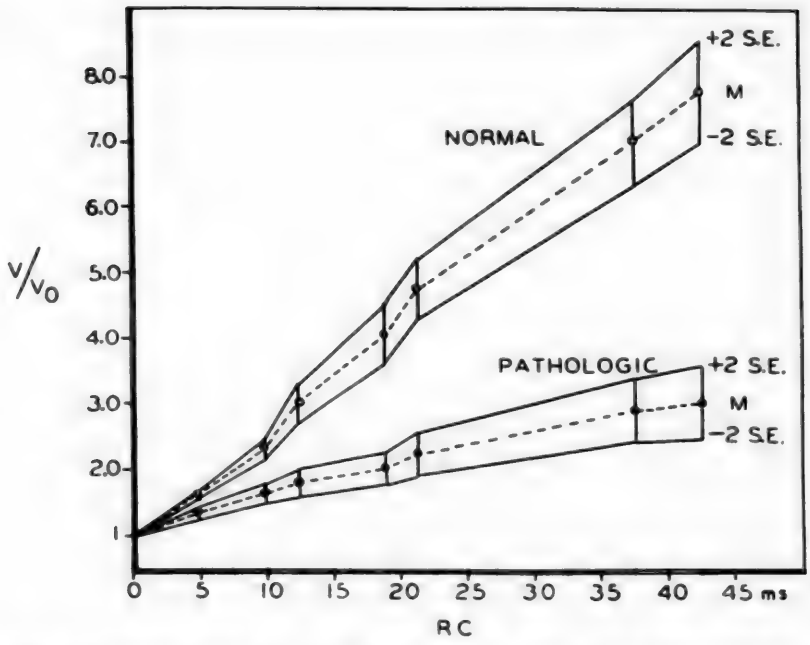


Fig. 4.—Threshold ratios (V/V_0) for different mean values ± 2 standard errors of the time-constants (RC), in normal and pathologic teeth.

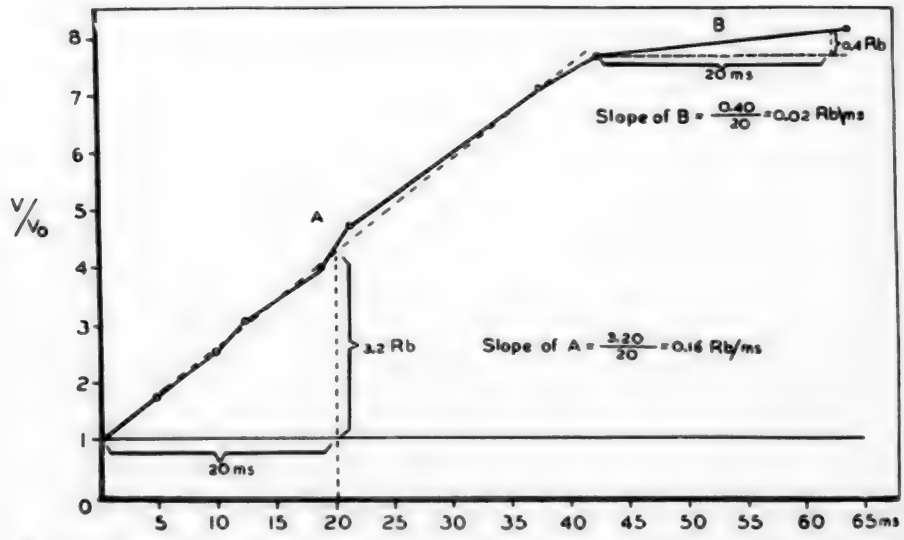


Fig. 5.—Graph representing the means of the threshold ratios of 5 normal cases as a function of time-constants. The "breakdown of accommodation" is clearly shown.

TABLE III
ACCOMMODATION OF PULP NERVES OF NORMAL CASES

CASE NO.	TOOTH NO.	AGE (YEARS)	SLOPE (RB/MS)
4	10	20	0.169
7	9	21	0.230
11	4	20	0.171
19	9	26	0.194
23	11	23	0.144
32	9	21	0.175
34	12	22	0.131
35	5	19	0.140
37	28	24	0.091
38	8	13	0.172
40	13	18	0.133
44	8	13	0.185
45	26	25	0.185
46	4	24	0.167
47	25	25	0.175
TOTAL = 15 CASES			0.164 ± 0.008 MEAN ± SE

currents with a time-constant greater than 42.5 ms is used. In 5 normal teeth, breakdown was studied using currents with time-constant up to 62.5 ms. In Fig. 5, the marked change in slope can be noted when the phenomenon of "breakdown" occurs; initially the slope was 0.16 rb/ms and after "breakdown" only 0.02 rb/ms. "Breakdown" occurred at a lower value than 42.5 ms in only 3 of the 15 normal subjects.

Pathologically Involved Teeth.—In these, the slope of the curve of accommodation has a significantly smaller slope ($P < 0.001$) than in normals, with a mean value of only 0.048 rb/ms (Table IV).

TABLE IV
ACCOMMODATION OF PATHOLOGIC PULP NERVES

CASE NO.	TOOTH NO.	AGE (YEARS)	SLOPE (RB/MS)
5	15	21	0.080
6	28	22	0.067
8	4	18	0.080
10	29	20	0.010
14	8	30	0.040
15	23	37	0.071
16	8	34	0.030
20	27	26	0.025
21	6	24	0.037
22	23	27	0.048
28	31	26	0.049
36	28	22	0.022
41	29	30	0.022
43	18	13	0.058
48	7	21	0.090
NUMBER OF CASES = 15			0.0486 ± 0.0066 MEAN ± SE

As in the normals, the increase of threshold is a linear function of the time-constants of the stimulating currents (Fig. 4). "Breakdown," however, does not occur regularly at one time-constant.

Comparison of Normal and Pathologic Cases.—Table V shows the values of the ratios of V/V_0 for normal and pathologic cases, their standard errors (SE),

and the values of the "t" test of statistical significance.[21] As can be noted, the ratios of the normal are significantly ($P < 0.001$) higher than those of the pathologic cases for every stimulating current.

TABLE V
STATISTICAL COMPARISON BETWEEN NORMAL AND PATHOLOGIC CASES

CURRENTS	NORMAL V/V_0	STANDARD ERROR (SE)	PATHOLOGIC V/V_0	STANDARD ERROR (SE)	VALUE OF "T"
E_1	1.66	0.038	1.37	0.051	4.56
E_2	2.33	0.080	1.64	0.083	6.00
E_3	3.02	0.150	1.81	0.110	6.50
E_4	4.09	0.230	2.04	0.126	7.82
E_5	4.75	0.230	2.24	0.160	8.96
E_7	7.02	0.330	2.92	0.240	10.00
E_{10}	7.78	0.390	3.03	0.280	9.90

In Fig. 4 the mean accommodation curves of normal and pathologic teeth are shown; each mean value is represented together with ± twice its standard error (SE).

Sensation Produced by the Stimulus.—All subjects studied described the sensation produced by the stimulating current as a fine prick, which is not painful. This finding is contrary to Brookhart's[23] contention, that only painful sensations can arise from a tooth.

DISCUSSION

Ohm Resistance of Teeth.—We have been unable to find a paper describing the progressive decrease of tooth resistance found in our experiments. This decrease may be due to the impregnation of the superficial layers of the enamel by the electrolytes of the conducting paste. A fall of resistance has been shown to occur in isolated pieces of enamel when these are impregnated with salts (Klein; cit. Leicester[24]). This process can be accelerated in vitro by the cataphoretic action of electric currents.[24]

It is improbable that the variations in resistance described in this paper are due to changes of resistance of the soft tissues interposed between tooth and forearm, which fluctuate between 15,000 and 30,000 ohms.[20] Resistances as low as these can have no influence in the notable drop of about 400,000 ohms (mean value) observed in this study. Therefore, it is probable that these changes can be ascribed to variations of conductance of the hard tissues of the teeth, as a consequence of the impregnation of this tissue by the electrolytes of the conducting paste.

Accommodation.—The high accommodation of normal dental pulp nerves (0.164 rb/ms) is worthy of note, for this value is higher than that of the sensitive and motor nerves measured by Kugelberg[16] in man. He described a "breakdown of accommodation" with stimuli whose threshold was 1.5 to 2.0 times rheobase in tactile fibers and 2.5 to 5.0 times rheobase in motor fibers; while in normal dental pulp, nerve "breakdown" does not occur except under stimuli whose threshold is greater than 7.5 rheobase. The values of the slope

of accommodation obtained by us are similar to those found by Concha[2] in the isolated nerve of Bufo chilensis, which has a slope of 0.26 rb/ms and shows "breakdown" with stimuli whose threshold is 6.0 times rheobase.

We have not found a normal tooth which shows "breakdown" at 1.5 rheobase as described by Björn.[20] Only in pathologic cases have we observed "breakdown" to occur at such low values.

High accommodation of pulp nerve fibers cannot be ascribed to combined stimulation of different sensitive elements in, or outside, the pulp; for a linear relationship between threshold and time-constant would not exist if this were the case. On the other hand, the sensations felt by the subjects were the same throughout the process of measurement. In experiments with pulpless teeth, we have observed that periodontal receptors do not respond to voltages as low as those which stimulated the pulp, and that the sensation of pressure produced by stimulation of the gingiva can be clearly differentiated from that produced by pulp stimulation.

The accommodation of the dental pulp is the highest observed in man, and is another demonstration that each excitable tissue has its own characteristics which depend on the speed of the reactions of its membrane. The tissues with fastest reactions are those which can re-establish membrane equilibrium more quickly, thus counteracting stimuli which tend to produce depolarization. It is probable that tissues with high accommodation have great adaptability to changes in their environment, while those tissues with naturally (heart, smooth muscle) or pathologically (damaged dental pulp) low accommodation are unable to counteract changes in their environment due to their slow processes of adaptation, and thus are able to respond to prolonged stimuli of constant intensity and show a tendency to spontaneous rhythmic discharge.

It is known that the nerves of the normal dental pulp which show a high accommodation react with difficulty to stimuli of long duration (heat, cold, osmotic variations, galvanic currents, etc.), that their responses only appear when there is an abrupt environmental change, and that they show exclusively "on and off" effects when excited with a prolonged stimulus. On the other hand, it is to be expected that damaged pulp nerves with low accommodation will show less adaptation and have a tendency to spontaneous and rhythmic discharges. As damage progresses and accommodation decreases, these fibers will become more and more sensitive to slow environmental change. The repetitive discharge which now appears, gives rise to pain by a process of central temporal summation.

This study shows that inflammation decreases accommodation of nerves as has also been demonstrated by Concha[25] in human skin. It is interesting to note that in inflammation, pulp nerves are similar in their accommodation characteristics to normal cardiac muscle and striated muscle after wallerian degeneration.

A finding of practical importance is the linear relationship between threshold of the pulp nerves and the time-constant of the stimulating currents, for

this means that accommodation can be studied by determining only 2 or 3 points of the curve, a method which simplifies measurement and which has been used successfully by several investigators in other tissues.[16-18]

The significant change of accommodation in the inflamed pulp presents a useful tool for diagnosis of pulpal alterations and confirms the results of Günther, Concha, and Röckel,[19] who studied teeth with normal and inflamed pulps, using only two exponentially increasing currents, and found a significantly smaller ratio of thresholds in the cases with pulpitis.

SUMMARY

The response of normal and inflamed dental pulps to exponentially increasing currents was studied. The ohm resistance of teeth was measured at short intervals and a significant decrease of resistance of about 53 per cent of the initial value, which occurs within a few minutes, noted. A linear relationship between threshold and the time-constants of the stimulating currents is described.

Normal dental pulps have high accommodation with a mean slope of 0.16 rheobase per millisecond rb/ms). Pulpitis decreases nerve accommodation significantly (slope 0.048 rb/ms).

The application of the study of accommodation to diagnosis of pulpal lesions is suggested. Due to the linear characteristics of the accommodation curve, only two points need be studied.

The author wishes to thank Prof. B. Günther, and Drs. J. Concha and G. Hodgson for their valuable assistance in several phases of this work.

REFERENCES

1. Nernst, W.: Zur Theorie des elektrischen Reizes, *Pflüger's Arch. ges. Physiol.* **122**: 280, 1908.
2. Concha, J.: Estimulación del Nervio Aislado de Batracio con Corrientes Eléctricas de Ascenso Exponencial. (Paper presented to Primera Jornada de Neurofisiologia, Santiago, Chile, 1954.)
3. Tasaki, I.: Electrical Excitation of the Nerve Fiber, *Jap. J. Physiol.* **1**: 1, 1950.
4. Diecke, F. P.: Accommodation in Single Nodes of Ranvier, *Fed. Proc.* **13**: 36 (118), 1954.
5. Lapicque, L.: Courants progressifs et Chronaxie, considérations générales, *Compt. rend. Soc. de biol.* **125**: **256**, 1937.
6. Solandt, D. Y.: A Comparison of Various Methods of Measuring the Time-Constant of Accommodation of Nerve, *Proc. Roy. Soc.* **B 119**: 335, 1936.
7. Kahn, E.: Untersuchungen über den Einfluss des Calciums auf die Wirkung der Muskelreizung mit konstantem Strom, *Pflüger's Arch. ges. Physiol.* **143**: 428, 1911.
8. Schriever, H., and Cebulla, R.: Ueber Erregbarkeitsänderungen des Nerven beim Uebergang von nicht-rhythmischer zu rhythmischer Reizbeantwortung, *Pflüger's Arch. ges. Physiol.* **241**: 1, 1938.
9. v. Kries, J.: Ueber die Abhängigkeit der Erregungs-Vorgänge von dem zeitlichen Verlaufe der zur Reizung dienenden Elektricitäts- Bewegungen, *Arch. Anat. Physiol.* **8**: 337, 1884.
10. Concha, J., and Donoso, M.: Curva de acomodación en la preparación neuromuscular aislada, *Rev. Chilena Neuropsiquiatría* **3**: 102, 1950.
11. Mena, E., and Concha, J.: Acomodación del músculo normal y denervado de rata. Acción de algunos anestésicos generales, *Bol. Soc. Biol.* **28**: 63, 1953.
12. Katz, B.: Neuro-muscular Transmission in Crabs, *J. Physiol.* **86**: 45P, 1936.
13. *Idem:* Multiple Response to Constant Current in Frogs' Medullated Nerve, *J. Physiol.* **88**: 239, 1936.
14. Liesse, A.: Chronaxie et accommodation, *Compt. rend. Soc. de biol., Paris* **127**: 831, 1938.

15. *Idem:* Influence de la circulation sur les constantes de temps de l'excitabilité électrique, *Compt. rend. Soc. de biol., Paris* **128**: 1193, 1938.
16. Kugelberg, E.: Accommodation in Human Nerves, *Acta physiol. scandinav.* **8**: 24, 1944.
17. Günther, B.: Nuevo método de electrodiagnóstico, *Rev. Chilena Neuropsiquiatría*, **3**: 99, 1950.
18. Weibel, J.: Influencia de la isquemia sobre el umbral y la acomodación nerviosa en el hombre, *Rev. Chilena Neuropsiquiatría* **3**: 101, 1950.
19. Günther, B., Concha, J., and Röckel, F.: Un nuevo método de electrodiagnóstico dental, *Bol. Soc. Biol.* **24**: 70, 1949.
20. Björn, H.: Electrical Excitation of Teeth, *Swedish Dent. J.* **39**: Supp., 1946.
21. Fisher, R. A.: Statistical Methods for Research Workers, London, 1950, Oliver and Boyd Edition.
22. Bernhard, C. G., Granit, R., and Skoglund, C. R.: The Breakdown of Accommodation. Nerve as Model Sense-Organ, *J. Neurophysiol.* **5**: 55, 1942.
23. Brookhart, J. M., Livingston, W. K., and Haugen, F. P.: Functional Characteristics of Afferent Fibers From Tooth Pulp of Cat, *J. Neurophysiol.* **16**: 634, 1953.
24. Leicester, H. M.: Biochemistry of the Teeth, St. Louis, 1949, The C. V. Mosby Co., p. 96.
25. Concha, J.: Personal communication.

DENTAL CONDITIONS AMONG THE POLYNESIANS OF PUKAPUKA (DANGER ISLAND)

II. The Prevalence of Periodontal Disease

G. N. DAVIES, D.D.S. (N. Z.)

Department of Preventive Dentistry, University of Otago Dental School, Dunedin, New Zealand

INTRODUCTION

THIS report is the second of a series covering the results obtained in a survey of natives living on the remote Pacific Atoll of Pukapuka. In an earlier paper[1] the geography of the island and the medical condition of the population was described.

METHOD

An attempt was made by clinical methods to assess both the prevalence of periodontal disease and the comparative effects of various etiological factors. The criteria used in the assessment of the degree of periodontal disease were as follows: NIL, No gingivitis in any region of the mouth. 1, Mild gingivitis localized to the interdental papillae or free gingival collar of 1 to 3 teeth. 2, Moderate gingivitis; extension of inflammation to form a continuous band along the marginal gingivae of more than 3 regions or teeth; slight detachment of the gingivae was a characteristic feature. 3, Severe gingivitis; extension of the inflammation to the attached gingivae with redness, swelling, edema, loss of stippling, loss of tonus and bleeding on palpation. 4, Severe gingivitis with associated generalized periodontitis.

Prevalence of Periodontal Disease.—Two hundred and twenty-five (45 per cent) of 497 subjects examined were affected to a greater or lesser extent by periodontal disease.

Prevalence of Various Degrees of Periodontal Disease.—In Table I the distribution of the various degrees of periodontal disease in Pukapuka is compared with similar data obtained in Rarotonga by Faine and Hercus.[2]

From these data it is apparent that, with the exception of its severe form, Grade 4, the incidence of periodontal disease is very similar in Pukapuka and Rarotonga. It is also apparent that the various degrees of involvement are fairly evenly distributed amongst the group affected.

Sex distribution of periodontal disease: Males and females are almost equally affected by this disease.

The application of Chi-square Test to the comparative data showed no significant difference between males and females (Chi-squared = 1.9369).

Received for publication March 17, 1955. Revised by author April 10, 1956.

TABLE I
INCIDENCE AND DISTRIBUTION OF VARIOUS DEGREES OF PERIODONTAL INVOLVEMENT IN PUKAPUKA AND RAROTONGA

DEGREE OF INVOLVEMENT	NUMBER AFFECTED		PERCENTAGE OF TOTAL NUMBER OF SUBJECTS		PERCENTAGE OF TOTAL NUMBER WITH CLINICAL SIGNS OF PERIODONTAL DISEASE	
	PUKA-PUKA	RARO-TONGA	PUKA-PUKA	RARO-TONGA	(225) PUKA-PUKANS	(142) RARO-TONGANS
No gingivitis	272	202	55	59	–	–
Mild gingivitis	62	50	12	15	28	35
Moderate gingivitis	68	39	14	11	30	28
Severe gingivitis	40	36	8	10	18	25
Severe gingivitis with associated generalized peridontitis	55	17	11	5	24	12
Totals	497	344	100	100	100	100

TABLE II
DISTRIBUTION OF PERIODONTAL DISEASE BY AGE GROUPS

AGE GROUP IN YEARS	DEGREE OF PERIODONTAL DISEASE					TOTAL	NUMBER AFFECTED	PERCENTAGE AFFECTED
	0	1	2	3	4			
1- 5	64	–	–	–	–	64	0	0.00
6-10	70	2	–	–	–	72	2	2.77
11-15	56	8	–	–	–	64	8	12.50
16-20	47	11	8	–	–	66	19	28.78
21-25	14	9	5	1	–	29	15	51.72
26-30	10	10	10	2	–	32	22	68.75
31-35	1	3	16	8	–	28	27	96.42
36-40	2	8	10	11	10	41	39	95.12
41 +	8	11	19	18	45	101	93	92.07
Totals	272	62	68	40	55	497	225	45.27

The relationship of age to the incidence and degree of periodontal disease: Data relating to age and periodontal disease are set out in Table II.

From these data it would appear that there is a strong association between increasing age and the incidence of periodontal disease. Children under 10 years of age rarely show signs of gingivitis. Mild gingivitis is fairly common in youths of 16-20, while the incidence of moderate to severe gingivitis increases rapidly with age from 21 years onwards to reach a peak at approximately 31-35 years of age.

Local Etiological Factors.—

1. *Functional aberrations:* Details were recorded of any of the following local functional factors which were judged to be contributing or exciting causes of periodontal disease. Occlusal trauma, nonocclusion, premature wear, abnormal occlusal habits, e.g., bruxism. These results are set out in Table III.

Occlusal trauma and premature wear appear to be the most important functional factors associated with periodontal disease. Lack of function (nonocclusion) was found in only 1.3 per cent of cases and abnormal occlusal habits were not detected.

2. *Food impaction:* Food impaction was found to be a local contributing factor in only 9.7 per cent of cases. It was most commonly found in association with mild gingivitis (Grade 1). Details are presented in Table IV.

TABLE III
FUNCTIONAL ABERRATIONS AND PERIODONTAL DISEASE

DEGREE OF INVOLVEMENT	NUMBER OF SUBJECTS	NUMBER AND PERCENTAGE OF SUBJECTS WITH:							
		OCCLUSAL TRAUMA		NONOCCLUSION		PREMATURE WEAR		ABNORMAL HABITS	
		NO.	%	NO.	%	NO.	%	NO.	%
1	62	27	43.5	2	3.2	3	4.8	0	–
2	68	24	35.2	1	1.4	7	10.2	0	–
3	40	11	27.5	0	–	3	7.5	0	–
4	55	16	29.0	0	–	5	9.0	0	–
Totals	225	78	34.6	3	1.3	18	8.0	0	0.0

TABLE IV
FOOD IMPACTION AND PERIODONTAL DISEASE

DEGREE OF PERIODONTAL INVOLVEMENT	NUMBER OF SUBJECTS	NUMBER OF SUBJECTS WITH FOOD IMPACTION	PERCENTAGE OF SUBJECTS WITH FOOD IMPACTION
1	62	13	20.9
2	68	0	0.0
3	40	1	2.5
4	55	8	14.5
Totals	225	22	9.7

3. *Calculus formation:* The amount of supra- and subgingival calculus present was graded according to the following criteria: *Supragingival calculus*—slight = confined to the crowns of lower anterior teeth in the vicinity of the gingival margin; moderate = covering most of the coronal surface of the lower 6 anterior teeth; severe = heavy deposit covering most of the coronal surfaces of more than 6 anterior teeth. *Subgingival calculus*—slight = Thin deposits confined to lower anterior teeth; moderate = slight deposits involving more than 6 anterior teeth; severe = heavy deposits involving more than 6 anterior teeth. The association between the extent of calculus formation and the various degrees of periodontal involvement is outlined in Tables V and VI.

It is apparent that there is a very significant association between the degree of periodontal disease and the amount of supragingival calculus (Chi-squared = 453, P = less than 0.001), and subgingival calculus (P = less than 0.001).

TABLE V
SUPRAGINGIVAL CALCULUS FORMATION AND PERIODONTAL DISEASE

DEGREE OF PERIODONTAL INVOLVEMENT	NUMBER OF SUBJECTS	DEGREE OF SUPRAGINGIVAL CALCULUS FORMATION							
		NIL.		SLIGHT		MODERATE		SEVERE	
		NO. AFF.	% AFF.	NO. AFF.	% AFF.	NO. AFF.	% AFF.	NO. AFF.	% AFF.
0	272	270	99.2	2	0.8	0	0.0	0	0.0
1	62	52	83.8	10	16.2	0	0.0	0	0.0
2	68	43	63.2	24	35.2	1	1.4	0	0.0
3	40	11	27.5	15	37.5	13	32.5	1	2.5
4	55	6	10.9	7	12.7	20	36.3	22	40.0
Totals	497	382	76.8	58	11.6	34	6.8	23	4.6

TABLE VI
SUBGINGIVAL CALCULUS FORMATION AND PERIODONTAL DISEASE

DEGREE OF PERIODONTAL INVOLVEMENT	NUMBER OF SUBJECTS	DEGREE OF SUBGINGIVAL CALCULUS FORMATION							
		NIL.		SLIGHT		MODERATE		SEVERE	
		NO. AFF.	% AFF.	NO. AFF.	% AFF.	NO. AFF.	% AFF.	NO. AFF.	% AFF.
0	272	270	99.2	2	0.8	0	0.0	0	0.0
1	62	44	70.9	18	29.1	0	0.0	0	0.0
2	68	15	22.0	35	51.4	17	25.0	1	1.4
3	40	1	2.5	3	7.5	26	65.0	10	25.0
4	55	1	1.8	2	3.6	13	23.6	39	70.9
Totals	497	331	66.5	60	12.0	56	9.3	50	10.0

The application of Chi-square test showed that the difference in calculus formation in males and females was not significant.

4. *Attrition:* An analysis of the data presented in Table VII reveals a strong association between the degree of attrition (as measured by the Index of Tooth Function)* and the degree of periodontal involvement. (Chi-squared = 102, P = less than 0.001).

TABLE VII
ASSOCIATION OF ATTRITION AND PERIODONTAL DISEASE

DEGREE OF PERIODONTAL INVOLVEMENT	NUMBER OF PERSONS WITH INDEX OF TOOTH FUNCTION OF			TOTAL
	0-0.9	1-1.9	2+	
0	107	16	2	125
1	31	21	3	55
2	19	35	7	61
3	6	24	6	36
4	12	19	10	41
	175	115	28	318

General Health Implications.—In a previous report[1] the author drew attention to the fact that sedimentation rates were high in those examined. Erythrocyte sedimentation rates tend to be increased in many acute and chronic infections. Table VIII was constructed in order to test whether any association existed between severe periodontal disease (Grades 3 and 4) and the sedimentation rate.

TABLE VIII
PERIODONTAL DISEASE AND ERYTHROCYTE SEDIMENTATION RATES

	NUMBER OF PERSONS WITH ERYTHROCYTE SEDIMENTATION RATES (MM. PER HOUR) OF				TOTAL
	0–	15–	30–	45–	
No periodontal disease or mild gingivitis (Grade 0 and 1)	18	39	114	106	277
Severe periodontal disease (Grades 3 and 4)	3	4	44	42	93
	21	43	158	148	370

*An Index of Tooth Function was computed for each subject in the following way: The degree of attrition on each individual tooth was recorded as 0 = normal contour – no facets, 1 = definite enamel facets, 2 = definite enamel facets with exposure of dentine, 3 = more advanced exposure of dentine with exposure of the recessional lines of the pulp, and 4 = actual pulp exposure. *The Index of Tooth Function* equals the sum of the attrition indices for each individual tooth divided by the number of teeth present.

The result of the application of Chi-squared test to these data reveals a significant association between severe periodontal disease and a high sedimentation rate. (Chi-squared = 8.5, $0.05>P>0.02$).

In the medical report of this expedition[3] data are presented which show an association between a chronic infection (filariasis) and blood serum protein levels. The estimations of total serum proteins were made on samples of blood by the copper sulfate specific gravity method of Phillips, van Slyke, Dole, Emerson, Hamilton, and Archibald[4] and Hoch and Marrack.[5] In Table IX serum protein values are compared with the presence of periodontal disease.

TABLE IX
PERIODONTAL DISEASE AND SERUM PROTEIN VALUES
(Expressed in terms of specific gravities)

	NUMBER PERSONS WITH SPECIFIC GRAVITY VALUES OF					TOTAL
	10200–	10280–	10290–	10300–	10310+	
No periodontal disease or mild gingivitis	29	55	70	79	44	277
Severe periodontal disease	6	10	17	37	23	93
	35	65	87	116	67	370

The results of the application of Chi-squared test to these data reveals a significant association between severe periodontal disease and high blood serum protein levels. (Chi-squared = 12.7, $0.02>P>0.01$).

The association between white blood cell counts and periodontal disease is shown in Table X.

TABLE X
LEUCOCYTE COUNTS AND PERIODONTAL DISEASE

DEGREE OF PERIODONTAL INVOLVEMENT	NUMBER OF PERSONS WITH LEUCOCYTE COUNTS PER ML. OF BLOOD OF					TOTAL
	2,000–	5,000–	8,000–	11,000–	14,000+	
0	5	101	88	18	13	225
1	8	35	16	3	0	62
2	12	41	12	3	0	68
3	3	20	16	1	0	40
4	5	30	15	2	2	54
Totals	33	227	147	27	15	449

From these data it would appear that a highly significant association exists between low leukocyte counts and periodontal disease. Since this finding is contrary to the generally accepted relationship,[6] the data were analyzed in greater detail. Table XI was constructed to show the mean leukocyte counts for groups with varying degrees of periodontal disease.

Statistical analyses of the differences of the various means reveal that a significant difference in leukocyte counts exists between those with no periodontal disease and those with periodontal disease of Grades 1 (S.E. diff. = 4.7), 2 (S.E. diff. = 5.9), 3 (S.E. diff. = 3.19), and 4 (S.E. diff. = 2.97).

There is no significant difference between Grades 1 and 2, Grades 2 and 3, and Grades 3 and 4.

TABLE XI
MEAN LEUCOCYTE COUNTS AND PERIODONTAL DISEASE

DEGREE OF INVOLVEMENT	NUMBER PERSONS	MEAN LEUKOCYTE COUNT	STANDARD DEVIATION
0	225	8,636	2,773
1	62	7,060	2,184
2	68	6,676	2,165
3	40	7,476	1,976
4	54	7,472	2,537
Totals	449	7,879	

These results are completely the reverse of those of Karshan, Tennenbaum, Karlan, and Leonard[6] who found that leukocyte counts were significantly higher in a group affected with periodontoclasia than in a group with no periodontal disease. In this study those with no periodontal disease had significantly higher leukocyte counts than those who did have periodontal disease.

Nutritional Factors Affecting the Periodontal Tissues.—The diet of the Pukapukans would appear to be somewhat deficient in vitamins A and C.[1] Both of these accessory food factors are important to the maintenance and integrity of epithelial and mesenchymal tissues. Deficiencies of vitamins A and C may thus predispose to periodontal disease.

DISCUSSION

The findings of this study confirm the fact that Pacific Island people generally are very susceptible to periodontal disease. The disease begins early in life and increases in severity with age.

Of the various local etiological factors the strongest association exists between subgingival and supragingival calculus formation and the degree of periodontal involvement. Calculus, once it has begun to form, accumulates progressively in lieu of any measures to control it by artificial methods of prophylaxis. In view of this fact, the high incidence of the severe forms of periodontal disease is not surprising.

No reasons for the high rate of calculus formation were elucidated. This problem is one worthy of further research. A thorough subgingival scaling was performed in a number of selected patients in the age range of 25 to 40 years. When this procedure was carried out on one side of the mouth only, a dramatic improvement in the color, tone, and consistency of the gingival tissues was manifest in all cases. After 48 hours the general appearance of the mouth was much improved and each patient commented on the "better feel and taste" of the mouth.

This suggests that the incidence of the severe forms of the disease could be reduced by the application of routine prophylaxis by trained personnel. In view of the effect of gross periodontal disease on erythrocyte sedimentation rates and blood serum protein levels, such a procedure would be a distinct advantage to the general health of the middle-aged Pukapukans.

The finding that leukocyte counts tended to be higher in those with no periodontal disease than in those with severe periodontal disease is interesting and may be indicative of the chronic nature of the severe forms of the disease.

Of the various general or systemic factors which may predispose to periodontal disease a demonstrable suboptimal intake of vitamins A and C must be of some significance, but its relative importance cannot be satisfactorily assessed on the evidence available.

SUMMARY

1. Of 497 subjects examined, 45 per cent (225) were affected to a greater or lesser extent by periodontal disease compared with 41 per cent of Rarotongans examined by Faine and Hercus.[2]

2. Of the 225 cases of periodontal disease 62 (28 per cent) were classed as mild gingivitis (Grade 1), 68 (30 per cent) as moderate gingivitis (Grade 2), 40 (18 per cent) as severe gingivitis, and 55 (24 per cent) as severe gingivitis with associated generalized periodontitis. These figures are similar to those for Rarotongans.

3. There was no significant difference in the incidence of periodontal disease between males and females.

4. A strong association was found between the incidence of periodontal disease and increasing age. Children under 10 years of age were rarely affected, whereas 92 per cent of adults over 41 years of age were affected.

5. Occlusal trauma, food impaction, and premature wear of teeth were found to be associated with periodontal disease in 34.6, 9.7, and 8 per cent of cases, respectively.

6. There was a very significant association between the degrees of periodontal involvement and the extent of both supra- and subgingival calculus formation.

7. No significant difference was found between calculus formation in males and females.

8. A strongly significant association was found between the degree of attrition of teeth and the degree of periodontal involvement.

9. Emphasis is laid upon the general health implications of severe periodontitis.

10. A significant association was found between high erythrocyte sedimentation rates and severe periodontal disease, and also between high serum protein values and severe periodontitis.

11. A negative association was shown to exist between leukocyte counts and severe periodontal disease.

12. The possible influence of vitamins A and C deficiencies as predisposing factors in periodontal disease is mentioned.

13. Some of the etiological factors are discussed and it is concluded that, in lieu of any known cause of a high rate of calculus formation, the application of routine subgingival scalings would do much to lower the incidence of severe periodontal disease on the atoll.

REFERENCES

1. Davies, G. N.: Dental Conditions Among the Polynesians of Pukapuka (Danger Island). I. General Background and the Prevalence of Malocclusion, *J. D. Res.* **35**: 115-131, 1956.
2. Faine, S., and Hercus, C. E.: The Nutritional Status of Cook Islanders, *J. Nutrition* **5**: 327-342, 1951.
3. Dempster, G. O. L.: Report on Medical Research Expedition; Pukapuka (Danger Island). Cyclostyled material. N. Z. Medical Research Council, 1951.
4. Phillips, R. A., van Slyke, D. D., Dole, V. P., Emerson, K., Hamilton, P. B., and Archibald, R. M.: Copper Sulphate Method for Measuring Specific Gravities of Whole Blood and Plasma, New York, 1945, Josiah Macy Jr. Foundation.
5. Hoch, H., and Marrack, J.: Estimation of Serum Proteins, *Brit. M. J.* **2**: 151-153, 1945.
6. Karshan, M., Tennenbaum, B., Karlan, F. R., and Leonard, H. J.: Blood Studies in Periodontoclasia. II, *J. D. Res.* **25**: 247-252, 1946.

CHEMICAL ANALYSIS OF HUMAN TEETH

KAMAL ASGAR

University of Michigan, School of Dentistry, Ann Arbor, Mich.

THE dental literature contains numerous articles concerning the chemical analysis of human teeth.[1-9] Most of this work was done in the early 1930's. Naturally, in these analyses the common chemical methods and procedures known at the time were used. Recently, a request was received for an analysis of some Greek teeth to compare their chemical compositions with that of domestic teeth. In recent years new methods in analytical chemistry have been developed which make the analysis of calcium, magnesium and phosphorus easier, faster, and more accurate. In this investigation some of these new methods have been applied. It is the purpose of this report to describe these methods and the compositions obtained.

Some investigators[1, 7] have shown that the composition of enamel and dentine of the teeth of one person may vary as much as that obtained from the teeth of several individuals. Others[2] have shown that even the difference between the chemical compositions of well-developed and hypoplastic enamel is of the same order as the difference between one individual and another. Thus, the sampling of human teeth for chemical analysis is quite important. In this analysis, 10 domestic and 10 Greek teeth were crushed separately and the results reported represent an average value for each group.

The human tooth is made up largely of inorganic material, and many investigators have analyzed only this portion of enamel and dentin. However, teeth contain organic as well as inorganic components, and some investigators, such as Deakins and Volker,[3] have reported the organic content of the enamel of human teeth. This report describes only the determination of the inorganic content of enamel and dentin.

After analyzing the inorganic part of human teeth, different investigators tried to assign a chemical formula to this structure. As a result, 3 rather important formulas have been given to tooth structure.

1. Tricalcium phosphate and calcium carbonate $Ca_3(PO_4)_2 + Ca\ CO_3$
2. Carbonate apatite $Ca_{10}(PO_4)_6\ CO_3$
3. Hydrozyl apatite $Ca_5(PO_4)_3OH + Ca\ CO_3$

The first formula is one that is rather old and outdated. The main point about it is the constant ratio between the phosphorus and calcium. Thewlis, Glock, and Murray[15] and Klement and Trömel[10] showed by x-ray methods of study that the phosphate of enamel is of an apatite nature. They

This study supported in part by a grant from the Office of Naval Research, Contract NR-180-360, to the University of Michigan, School of Dentistry.

Received for publication June 16, 1955.

showed that all lines in apatite appeared on the x-ray pattern of enamel. Hendricks, Hill, Jacob, and Jefferson,[11] Bale, Hodge, and Warren,[5] and Hodge and McKay[12] also gave the apatite configuration to human enamel.

Apatites are found in nature and have a general formula of $Ca_5(PO_4)_3X$, where X can be replaced by Cl, F, or OH. Magnesium, sodium, or potassium may replace calcium in the crystal lattice. The formula for carbonate apatite is based on the assumption that the CO_3 ion has replaced 2 X's from 2 molecules of apatite. However, some crystallographers, such as Trömel and Moller,[13] consider the existence of carbonate apatite impossible, and as a result this formula has lacked support. A few of the investigators, such as Klement and Trömel,[10] Bredig,[14] Thewlis,[15] and Bale[16] considered the main substance of the dental enamel to be hydroxyl apatite. Today, hydroxyl apatite is the accepted configuration given to the inorganic content of human teeth, and more work[17] using the powder x-ray method also shows this to be the best possible structure.

METHOD

Separation of Dentin From Enamel.—Human teeth after being cleaned and washed with soap and water were dried in an oven for two to three hours at 180° to 185° F. This removed all the water from the surface and made the teeth easy to pulverize. Dry teeth were placed in a mortar, crushed, and screened through 100-mesh screen. Oversize particles were returned to the mortar, recrushed, and rescreened until all the particles passed through 100-mesh screen.

The difference in the density of enamel and dentin makes the separation of these 2 substances rather simple.[18, 19] A mixture of 92 ml. of bromoform and 8 ml. of acetone (density 2.70) was used. About 1 Gm. of the pulverized teeth was placed in a centrifuge tube and about 7 ml. of the bromoform mixture was added to the powdered teeth sample. The tube was placed in the centrifuge and the rate of the spinning of the centrifuge was slowly increased to the maximum for the machine. After about seven to eight minutes the centrifuge was stopped and the tube was removed. Dentin having a density of 2.14 floats, while enamel with the density 2.89 to 3.00 settles to the bottom of the tube.

Dentin and enamel collected this way were not pure and had to be purified. For purifying the enamel, pure bromoform (density 2.85) was used. Again the enamel collected on the bottom. The pure liquid bromoform, having a higher density than the bromoform-acetone mixture, allows the small amount of dentin to float easily. In this way the enamel was purified. For purification of dentin, bromoform and acetone with the density of 2.42 were used (79 ml. bromoform, 21 ml. acetone). Dentin and cementum, having lower density than the liquid, floated on the surface, while enamel was collected on the bottom. Finally, a bromoform and acetone mixture with a density of 2.07 (62 ml. bromoform, 38 ml. acetone) was used for separation of dentin from cementum. Dentin collected on the bottom, while cementum (density 2.03) floated on the surface.

Extraction of Organic Part and Dissolving of Teeth.—Organic parts of enamel and dentin were extracted by the modified Gabriels method which was discussed briefly by Crowell, Hodge, and Line.[7] By using 3 per cent solution of KOH in ethylene glycol, the organic contents of enamel and dentin were extracted, leaving the inorganic residue in the flask. The inorganic residue of enamel and dentin, after being washed with distilled water for a few times, was dried and weighed.

A known amount of the inorganic part of enamel and dentin was dissolved in nitric acid and was transferred into 100 ml. volumetric flasks. These solutions were used in determining the phosphorus content of enamel and dentin.

Phosphorus Determination.—The prepared solution as mentioned above was used for the determination of phosphorus. Phosphorus was precipitated as ammonium molybdiphosphate, which is usually called a yellow precipitate. This precipitate is pure and theoretical only when it is formed under exact conditions. The facts which must be considered in precipitating phosphorus as ammonium molybdiphosphates are: (1) All phosphorus must be present as orthophosphoric acid—H_3PO_4, (2) A certain excess of free nitric acid must be present and a large excess of it must be avoided, (3) The presence of a large amount of ammonium nitrate is necessary, and (4) The precipitate should be made from a hot solution at 65°C., and should not be allowed to stand at this temperature more than fifteen minutes.

Preparation of Molybdic Acid Solution.—To 50 Gm. of pure molybdic anhydride, MoO_3, 50 ml. of concentrated ammonia and 150 ml. of water were added. The mixture was heated gently until all of the molybdic acid was dissolved. At this point the solution was checked by smelling for the presence of ammonia. Some ammonia was added until the distinct ordor of ammonia was noticeable. About 250 ml. of concentrated nitric acid was diluted to 600 ml. Both of the solutions were cooled and mixed by pouring the molybdate solution into the diluted nitric acid slowly with constant stirring. More water was added until the total volume was 1 liter.

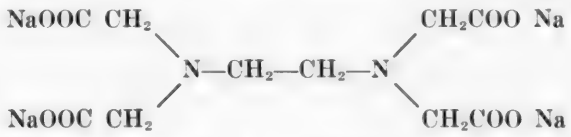
Precipitation of Ammonium Molybdiphosphate.—By means of a pipette, 25 ml. from the solution of teeth dissolved in nitric acid was drawn off. Approximately 50 ml. of fresh molybdic acid solution was heated to nearly 60°C. and added slowly with constant stirring to the teeth solution which was previously heated to 60°C. The mixture was put aside for two hours at room temperature until the precipitation was completed. After the precipitation was completed, it was filtered through a Gooch crucible. The precipitate was dried at 120° to 130°C. and weighed. The residue contained 1.64 per cent of phosphorus. Since the residue is rather hygroscopic, it must be weighed quickly.

The results of 2 runs made by this method were as follows:

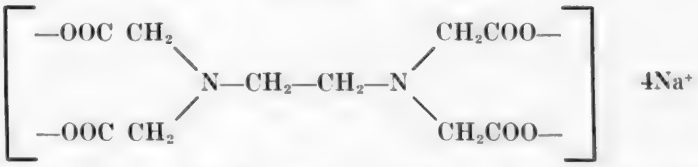
PERCENTAGE OF PHOSPHORUS

	DOMESTIC		GREEK	
	DENTIN (PER CENT)	ENAMEL (PER CENT)	DENTIN (PER CENT)	ENAMEL (PER CENT)
Sample I	13.15	16.38	12.56	14.47
Sample II	13.36	16.37	12.46	14.69

Determination of Calcium and Magnesium.—Calcium and magnesium in dentin and enamel were determined by titration with Versene.* The chemical structure of this compound is usually presented as



Since it is a salt of a strong base, it is completely ionized in solution. Consequently, its structure may also be presented by the formula



which could be contracted to Na_4Ve. The sodium and other alkali metal salts of ethylenediaminetetraacetic acid are quite soluble in water. The acid itself is insoluble. Thus, the addition of strong acid to sodium versenate results in the formation of a crystalline precipitate of the acid.

Versene reacts with alkaline earth and heavy metals to form an unusually stable soluble metal chelate compound. The chemical reactions which take place can be shown as follows:

$$Ca^{++} + Na_4Ve \rightarrow Na_2(CaVe) + 2Na^+$$

In titration of calcium with Versene, the Versene indicator was used. The indicator is blue in color but forms a red color with the magnesium ion and stays blue in the presence of calcium ion. The calcium ion reacts with Versene completely before the magnesium ion is being attacked. With this method, therefore, calcium and magnesium ions will be titrated as a total. Having the magnesium ion in the solution, the indicator will have a red color which will be converted to a bluish purple at the end point. In order to separate the amount of magnesium from that of calcium, calcium was precipitated, filtered, redissolved, and titrated separately. The amount of magnesium was found by the difference. When calcium is titrated separately, in order to have a sharp end point, a known concentration of the magnesium ion was added.

For the best results in these titrations, the pH of the solution should be about 10.[21] With the presence of the phosphorus ion, however, at pH of 10, calcium will form calcium phosphate in which case the end point may not be sharp. Therefore, an ion exchange technique was applied to separate the phosphorus ion from that of calcium.

Principle of Separation.—After dissolving the sample in hydrochloric acid, the solution was passed through a column containing a cation exchange resin.[22-24] The resin was in the hydrogen form on which the metal cations in the solution

*Versene is the aqueous solution of the tetra sodium salt of ethylenediaminetetraacetic acid made by the Bersworth Chemical Company of Framingham, Mass.[20]

were retained, being thus separated from the phosphoric acid which passes quantitatively through the column. After the column was washed with water, approximately 4 normal hydrochloric acid solution was passed through the column. The acid solution carries down all the metal cations from which calcium and magnesium were determined.

Preparation of Standard Versenate Solution.—About 10 Gm. of disodium-dihydrogenethylenediaminetetraacetate dihydrate* and 2 Gm. of sodium hydroxide pellets were dissolved in water and made up to one liter. This solution was standardized with standard calcium chloride solution which was made by dissolving a known amount of calcium carbonate in hydrochloric acid.

The actual values obtained by this method are:

PERCENTAGE OF CALCIUM AND MAGNESIUM

	DOMESTIC		GREEK	
	DENTIN (PER CENT)	ENAMEL (PER CENT)	DENTIN (PER CENT)	ENAMEL (PER CENT)
Sample I (Ca + Mg)	30.97	37.20	27.25	33.47
Sample II (Ca + Mg)	31.02	37.05	27.22	33.25
Average (Ca + Mg)	31.00	37.12	27.24	33.37
Sample I Ca	30.36	36.16	26.73	32.87
Sample II Ca	30.14	36.15	26.89	32.79
Average Ca	30.25	36.16	26.81	32.83
Average Mg	0.75	0.96	0.43	0.54

Determination of Carbonate Ion in Teeth.—Enamel and dentin samples, after being weighed, were placed on a special gas-tight apparatus. The success of determination of carbonate ion largely depends upon construction of a compact gas-tight train. The apparatus used for this purpose was formed of a wide mouth decomposition flask which was supported on a wire gauze on a ring stand. The flask was closed with a 2-hole rubber stopper containing a calcium chloride tube filled with "ascarite" (sodium hydroxide asbestos absorbent). By means of a 6 mm. diameter piece of glass tubing, the condenser was connected to a U-form tube. Two of these U-form tubes were employed and were supported with a copper wire from an iron rod which was held by two ring stands. The first U-tube served to absorb water vapor that escapes from the condenser as well as to remove any hydrochloric acid or hydrogen sulfide from the gas. The first third of the first U-tube was filled with anhydrous copper sulfate which served to remove any hydrogen sulfide, and the last two thirds with dehydrite $Mg(ClO_4)_2$, which is an effective absorbent of water. The second U-tube was filled with the drying agent, dehydrite. From the second U-tube a suitable shaped glass tube led to the special absorption bulb which was filled with fresh ascarite on top of which was placed a layer of drying agent, about 1 cm. thick. From the absorption bulb a tube led to another bulb containing concentrated sulfuric acid. This bulb served both to indicate the rapidity of the gas flow through the train and to prevent any water vapor from entering the train from the suction end. The last bulb in

*From Bersworth Chemical Company.

the train was an empty one which acted as a trap to catch any water that might be drawn back from the suction. This bulb was connected to the suction by heavy walled rubber tubing.

Before running the actual determination, the train was tested for any possible leakage. A known amount of calcium carbonate was placed in the decomposition flask, and through a separatory funnel hydrochloric acid was added very slowly. The hydrochloric acid decomposed the calcium carbonate, and suction pulled the formed gases through different tubes and bulbs as described. Eventually, carbon dioxide was absorbed in the absorption bulb and the amount of it was found by weighing the bulb before and after each experiment. In the absorption bulb the following chemical reaction takes place:

$$CO_2 + 2NaOH \rightarrow Na_2CO_3 + H_2O$$

The water vapor formed from this reaction will be absorbed by the drying agent which was placed on the top of the ascarite in the same bulb. Therefore, the change of weight of this bulb is due to 2 factors: (1) Conversion of 2NaOH to $NaCO_3$, and (2) for every mole of CO_2, one mole of water will be formed which would be absorbed in the same bulb also.

The results of runs made by this method are:

PERCENTAGE OF CO_2

	DOMESTIC		GREEK	
	DENTIN (PER CENT)	ENAMEL (PER CENT)	DENTIN (PER CENT)	ENAMEL (PER CENT)
CO_2 in Sample I	3.33	4.95	3.47	2.43
CO_2 in Sample II	3.74	4.65	3.56	2.11

Other Elements Present in the Teeth.—Human teeth contain besides calcium, phosphorus, magnesium, and carbonate ions, which are the major ingredients, other elements in a very low concentration. With usual chemical analysis, these elements cannot be detected. Therefore, spectroscopic analysis was applied in order to identify these minor elements. It was found that domestic dentin and enamel have the same elements as Greek dentin and enamel, but with different concentrations. At the moment, the quantitative spectroscopic analysis for these elements has not been completed. By this method the qualitative presence of the following elements with a low concentration were identified: Pb, Zn, Si, Ag, Cu, Fe, Al, Sn, Na, Cl, F.

RESULTS OF THE CHEMICAL ANALYSIS OF TEETH

PER CENT OF ELEMENTS	DOMESTIC TEETH		GREEK TEETH	
	DENTIN	ENAMEL	DENTIN	ENAMEL
Ca	30.25	36.16	26.81	32.83
Mg	0.75	0.96	0.43	0.54
P	13.25	16.37	12.51	14.58
CO_2	3.54	4.80	3.51	2.27
Ca/P	2.28	2.19	2.14	2.26

SUMMARY

Greek and domestic teeth were analyzed and the results are reported. In this analysis dentin was separated from enamel by the Manly and Hodge

method.[19] Phosphorus was determined by precipitating it as ammonium molybdiphosphate which is usually called the yellow precipitation method. Calcium and magnesium were determined by titrating with Versene[20, 21] at a pH of about 10 from phosphorus free solution. In this solution phosphorus was removed by means of ion exchange column.[22-24] The carbonate ion is reported as CO_2. By adding hydrochloric acid, CO_2 was released and absorbed by sodium hydroxide. A special apparatus was used for this determination. For the qualitative determination of other elements present in teeth at very low concentrations, spectroscopic analysis was used.

REFERENCES

1. Armstrong, W. D., and Brekhus, P. J.: Chemical Constitution of Enamel and Dentine, *J. Biol. Chem.* **120**: 677, 1937.
2. Bowes, J. H., and Murray, M. M.: The Variations in Chemical Composition in Relation to Dental Structure, *Biochem. J.* **30**: 977, 1936.
3. Deakins, M., and Volker, J. F.: Amount of Organic Matter in Enamel From Several Types of Human Teeth, *J. D. Res.* **20**: 117, 1941.
4. Steadman, L. T., Hodge, H. C., and Horn, H. W.: Spectrochemical Studies of Potassium in Bone and Tooth Substance, *J. Biol. Chem.* **140**: 71, 1941.
5. Bale, W. F., Hodge, H. C., and Warren, S. L.: Roentgen Ray Diffraction Studies of Enamel and Dentine, *Am. J. Roentgenol.* **32**: 369, 1934.
6. Sobel, A. E., and Sklersky, J.: A Direct Acidimetric Microtitration Method for Calcium, *J. Biol. Chem.* **122**: 665, 1938.
7. Crowell, C. D., Hodge, H. C., and Line, W. R.: Chemical Analysis of Teeth Samples Composed of Enamel, Dentine, and Cementum, *J. D. Res.* **14**: 251, 1934.
8. LeFevre, M. L., Bale, W. F., and Hodge, H. C.: The Chemical Nature of the Inorganic Portion of Fetal Tooth Substance, *J. D. Res.* **16**: 85, 1937.
9. LeFevre, M. L., and Hodge, H. C.: Chemical Analysis of Tooth Sample Composed of Enamel, Dentine, and Cementum, *J. D. Res.* **16**: 279, 1937.
10. Klement, R., and Trömel, G.: Hydroxylapatite, der Hauptbestandteil der anorganischen Knochen und Zahnsubstanz, *Ztschr. phys. Chem.* **213**: 263, 1932.
11. Hendricks, S. B., Hill, W. L., Jacob, K. D., and Jefferson, M. E.: Structural Characteristics of Apatite-Like Substances and Composition of Phosphate Rock and Bone as Determined From Microscopical and X-ray Diffraction Examinations, *Ind. Eng. Chem.* **23**: 1413, 1931.
12. Hodge, H. C., and McKay, J. H.: The Microchardness of Teeth, *J. A. D. A.* **20**: 227, 1933.
13. Trömel, G., and Moller, A.: Die Bildung schwer löslicher Kalziumphosphate aus wässriger Lösung und die Beziehungen dieser Phosphate zur Apatitgruppe, *Ztschr. Anorg. Chem.* **206**: 227, 1932.
14. Bredig, M. A.: Zur Apatitstruktur der anorg. Knochen- und Zahnsubstanz, *Ztschr. phys. Chem.* **216**: 239, 1933.
15. Thewlis, J., Glock, G. E., and Murray, M. M.: Chemical and X-ray Analysis of Dental, Mineral and Synthetic Apatites, *Trans. Far. Soc.* **35**: 358, 1939.
16. Bale, W. F.: A Comparative Roentgen-Ray Diffraction Study of Several Nature Apatites and the Apatite-Like Constituent of Bone and Tooth Substance, *Am. J. Roentgenol.* **43**: 375, 1940.
17. Tovborg, J. A., and Moller, A.: Determination of Size and Shape of the Apatite Particles in Different Dental Enamels and Dentin by the X-Ray Powder Method, *J. D. Res.* **27**: 524, 1948.
18. Brekhus, P. J., and Armstrong, W. D.: A Method for the Separation of Enamel, Dentine, and Cementum, *J. D. Res.* **15**: 23, 1935.
19. Manly, R. S., and Hodge, H. C.: Density and Refractive Index Studies of Dental Hard Tissues, *J. D. Res.* **18**: 133, 1939.
20. Abeshouse, B. S., and Weinberg, T.: Experimental Study of Solvent Action of Versene on Urinary Calcium, *J. Urol.* **65**: 2, 1951.
21. Plumb, R. C., Martell, A. E., and Berswork, F. C.: Series of Metal Complexes of Ethylene Diamine Tetra Acetic Acid. Presented at the Oil Chemists Convention, Oct. 31, 1949, Chicago, Ill.
22. Samuelson, D.: Ion Exchange in Analytical Chemistry, New York, 1953, John Wiley & Sons, Inc.
23. Goudie, A. J., and Rieman, W. M.: Determination of Phosphate by Ion Exchange, *Anal. Chem.* **24**: 1067, 1952.
24. Kindt, B. H., Balis, E. W., and Liebhafsky, H. A.: Alkalimetric Determination of Phosphate After Separation of Calcium by Ion Exchange, *Anal. Chem.* **24**: 1501, 1952.

A STUDY OF TIN IN ENAMEL

FINN BRUDEVOLD AND LUVILLE T. STEADMAN

Eastman Dental Dispensary and School of Medicine and Dentistry of the University of Rochester, Rochester, N. Y.

THE American adult intake of tin averages about 17 mg. per 24 hours,[1] and yet extremely small quantities are retained in the body. Kehoe, Cholak, and Story[1] found only 0.8 ppm in long bones and smaller amounts in other tissues. Systemic absorption appears to be virtually absent even in cases of heavy exposure to tin oxide by inhalation, although large quantities of tin are deposited and retained in the lungs.[2] Tin has been detected spectrographically both in enamel and dentin,[3] but no estimation of the amounts which may be present has been reported. From the fact that only minute quantities are deposited in other tissues, it might be predicted that small amounts, if any, are laid down in tooth structure prior to eruption. However, there is some reason for believing that tin may be picked up to a greater extent by the external enamel after eruption. Muhler and his associates have found that stannous fluoride is more effective than sodium fluoride both in decreasing the solubility of powdered enamel[4-7] and in reducing dental caries in rats.[7-10] In addition, Manly and Bibby[11] observed that stannous chloride reduces the solubility of powdered enamel, and recently Ericsson[12] reported that both tin chloride and tin fluoride will produce a similar effect on the intact enamel surface. More significant is the finding that stannous fluoride applied topically will produce a greater reduction in dental caries in children than sodium fluoride,[13] and there is also some evidence that stannous fluoride incorporated in a tooth paste may be similarly effective.[14] These findings suggest that tin as stannous fluoride will react with and perhaps be picked up by the surface enamel. Attempts to study the nature of the reaction involved have not been conclusive. According to Fisher, Muhler, and Wust,[15] it appears that stannous fluoride produces an amorphous material on the surface of enamel particles which becomes crystallized upon heating. However, they found that this material is not readily produced by stannous chloride. Therefore, it is believed that stannous and fluoride ions will interact to bring about an enhanced reaction with the enamel.

From these studies it appears that tin salts may prove to be important therapeutic agents in combating dental caries. Since tin forms insoluble phosphate, it is possible that tin may be taken up normally by the enamel, and that it, like fluoride, may accumulate in the outer enamel and thus produce a caries-reducing effect. The possibility of tin acquisition was investigated by determining the concentrations of tin in surface and subsurface layers of enamel from teeth of different ages.

This work was supported in part by the Bristol Myers Company.
Received for publication April 5, 1955.

METHOD

Pooled samples of enamel representing successive layers of enamel from teeth of different ages were obtained by grinding with a diamond stone according to a method previously described.[16] Sufficiently large samples were collected to permit determinations of fluoride, lead, and copper, in addition to tin. The findings on the first 3 of these elements have been published in separate papers and a description of the tooth material used was given in the paper on fluoride.[16] Included in the present study are determinations on 4 successive enamel layers from partially and fully developed unerupted teeth and from erupted teeth of persons in the age groups under 20, 20 to 29, 30 to 49, and over 50 years of age. Since a great number of the erupted teeth had been filled with amalgam, which is known to contain large amounts of tin, precautions were taken to include only intact teeth in the group over 50 years of age. Therefore, this group served as a control to estimate the possible effect of dental restorations on the presence of tin in the enamel of erupted teeth from the other age groups.

Tin was measured by a spectrographic method employing the d.c. carbon arc and a Bausch and Lomb medium quartz spectrograph. The tooth material was added dry in amounts of 2 to 10 mg. to the crater of regular grade spectrographic electrodes and arced for three minutes at a current of 15 amperes and a line potential of 130 volts. The tin line 2840.0 A.U. was read and compared with platinum 2650.9 A.U. as the internal standard, 40 μg of which was previously added to the negative sample electrode. Relative intensities were determined by a rotating sector method. Analyses generally were done in sextriplicate and the tin value obtained is believed to be accurate to $\pm$ 15 per cent.

RESULTS

The results are given in Table I. There is a conspicuous difference in the amount of tin found in the unerupted teeth, in the teeth from persons over 50 years of age and the mottled teeth on the one hand, as compared with that found in the remaining erupted teeth on the other. The former groups, which

TABLE I

CONCENTRATION OF TIN IN PARTS PER MILLION IN SUCCESSIVE LAYERS OF ENAMEL

LAYER NO.	UNERUPTED		ERUPTED				
			FILLED			INTACT	
	I*	II†	<20	20-29	30-49	>50	MOTTLED
1	0.5	1.5	20.0	65	80	7.0	3.5
2	0.5	1.5	4.0	21	22	2.0	2.0
3	0.5	1.5	2.5	28	Lost	0.0	0.5
4	0.5	1.0	2.0	17	16	0.0	1.0

*Partly developed.
†Fully developed.

represented only intact teeth, gave low tin values, the concentrations ranging from 0.5 ppm in the outer enamel of the partly developed unerupted teeth to 7.0 ppm in the teeth from persons over 50 years of age. The groups of erupted teeth which included filled teeth gave higher values, the concentrations in the

outer enamel ranging from 20 ppm in the under 20 years group to 80 ppm in the 30 to 49 years group. In all the erupted teeth, including the mottled teeth, the greatest amount of tin was found in the outermost enamel layer, and decreasing amounts were found in the successive layers of subsurface enamel. In unerupted teeth, tin concentrations were the same in surface and subsurface enamel.

DISCUSSION

Our findings demonstrate that a distinction must be made between the small amounts of tin which are normally laid down in the enamel and the tin which may be incorporated from dental filling materials. The infinitesimal pre-eruptive uptake is in accord with reports of low levels of tin in other tissues. The minute amounts of tin in the enamel of mottled teeth and of intact teeth from the over 50 years group show that posteruptive deposition of tin from saliva, water, or food is also of small magnitude. Obviously the amount of tin in enamel is too small under normal conditions to be of any significance.

The relatively high amounts of tin in the groups of teeth which included filled teeth must be related to the presence of amalgam restorations. Since all fillings were removed before enamel samples were obtained, it is doubtful that contamination of our samples with amalgam contributed to our high tin values. A more likely possibility is inclusion of tin from amalgam restorations as a result of diffusion or incorporation of metallic components into surrounding enamel. Massler and Barber[17] found that such a diffusion may occur readily in dentin and that it may also take place in enamel, but to a smaller extent. In this study, a spectrographic analysis revealing the presence of tin and copper was only done on discolored dentin, but microscopic examination of discolored enamel also showed the presence of metal-like particles in the structure. Therefore, this type of diffusion may have contributed to our high tin values. In addition, tin may have been incorporated into the surface enamel from amalgam restorations because of mastication, toothbrushing, or polishing. The frequent occurrence of secondary caries at the margins of amalgam fillings suggests that tin originating from amalgam has no caries-reducing effect.

The reduction in surface solubility produced by stannous chloride and stannous fluoride, and the caries-reducing effect of the latter compound when applied topically, are of considerable interest in view of our findings that tin is not normally taken up by the enamel. Since the above tin salts have been found to be most effective at a low pH, it is possible that tin may be acquired only under acid conditions. It is also possible that the decrease in solubility is caused by the deposition on the enamel surface of the insoluble precipitate described by Fisher, and that the superior effectiveness of tin fluoride over sodium fluoride in reducing dental caries is attributable to this and other reactions rather than increased deposition of tin in the enamel.

SUMMARY

1. The amount of tin has been determined in successively ground layers of enamel from groups of unerupted and erupted teeth of different ages.

2. In the enamel of unerupted teeth, tin was found to be present in amounts of 1 ppm or less, and to be evenly distributed in depth.

3. There is no appreciable posteruptive uptake of tin by the external surface of intact teeth. Only 7 ppm of tin was found in the external enamel of intact teeth from persons over 50 years of age. No tin could be detected in the bulk of the enamel of these teeth.

4. The tin content in the enamel of teeth with amalgam restorations was greater than that in the enamel of intact teeth. It is believed that the increased tin content is caused by incorporation of metallic components from amalgam fillings into adjacent enamel.

REFERENCES

1. Kehoe, R. A., Cholak, J., and Story, R. V.: A Spectrochemical Study of the Normal Ranges of Concentration of Certain Trace Metals in Biological Materials, *J. Nutrition* **19**: 579, 1940.
2. Dundon, C. C., and Hughes, J. P.: Stannic Oxide Pneumoconiosis, *Am. J. Roentgenol.* **63**: 797, 1950.
3. Lowater, F., and Murray, M. M.: Chemical Composition of Teeth. V. Spectrographic Analysis, *Biochem. J.* **31**: 837, 1937.
4. Muhler, J. C., and Van Huysen, G.: Solubility of Enamel Protected by Sodium Fluoride and Other Compounds, *J. D. Res.* **26**: 119, 1947.
5. Muhler, J. C., Boyd, T. M., and Van Huysen, G.: Effect of Fluorides and Other Compounds on the Solubility of Enamel, Dentin and Tricalcium Phosphate in Dilute Acids, *J. D. Res.* **29**: 182, 1950.
6. Muhler, J. C., and Day, H. G.: Relation of pH to the Effectiveness of Sodium Fluoride and Stannous Fluoride in Decreasing Enamel Solubility, *J. D. Res.* **31**: 102, 1952.
7. Muhler, J. C., Nebergall, W. H., and Day, N. G.: Studies on Stannous Fluoride and Other Fluorides in Relation to the Solubility of Enamel in Acid and the Prevention of Experimental Dental Caries, *J. D. Res.* **33**: 33, 1954.
8. Muhler, J. C., and Day, H. G.: Effects of Stannous Fluoride, Stannous Chloride and Sodium Fluoride on the Incidence of Dental Lesions in Rats Fed a Caries-Producing Diet, *J. A. D. A.* **41**: 528, 1950.
9. Muhler, J. C., Nebergall, W. H., and Day, H. G.: Preparations of Stannous Fluoride Compared With Sodium Fluoride for the Prevention of Dental Caries in the Rat, *J. A. D. A.* **46**: 290, 1951.
10. Muhler, J. C., and Day, H. G.: Effect of Stannous Fluoride in Food and in Drinking Water on Caries Prevention in Rats on High Sucrose and Coarse Corn Diets, *J. Nutrition* **44**: 413, 1951.
11. Manly, R. S., and Bibby, B. G.: Substances Capable of Decreasing the Acid Solubility of Tooth Enamel, *J. D. Res.* **28**: 160, 1949.
12. Ericsson, Y.: Reduction of the Solubility of Enamel Surfaces, Acta. Odont. scandinav. **9**: 60, 1950-1951.
13. Howell, C. L., Gish, C. W., Smiley, R. D., and Muhler, J. C.: Effect of Topically Applied Stannous Fluoride on the Dental Caries Experience in Children, *J. A. D. A.* **50**: 14, 1955.
14. Muhler, J. C., Radike, A. W., Nebergall, W. H., and Day, H. G.: Effect of a Stannous Fluoride Containing Dentifrice on Caries Reduction in Children. Caries Experience After One Year, *J. A. D. A.* **50**: 163, 1955.
15. Fisher, R. R., Muhler, J. C., and Wust, C. J.: Effects of Several Fluoride Reagents on the Surface Structure of Powdered Dental Enamel, *J. D. Res.* **33**: 50. 1954.
16. Brudevold, F., Gardner, D. E., and Smith, F. A.: The Distribution of Fluoride in Human Enamel, *J. D. Res.* **35**: 420, 1956.
17. Massler, M., and Barber, T. K.: Action of Amalgam on Dentin, *J. A. D. A.* **47**: 415, 1953.

ESTIMATION OF THE CHLORIDE LEVEL OF SALIVA

JOHN T. ANDERS

Biochemistry Laboratory, Forsyth Dental Infirmary for Children, Boston, Mass.

A GREAT interest has been displayed over the last 8 to 10 years regarding the effect of fluoride on the incidence and rate of tooth decay. It was partially as a result of those investigations that this study on the other halogens was contemplated. The investigation presented here deals specifically with the presence in saliva of the chloride ion and its possible effect on tooth decay. A study of available literature reveals that very limited work on chloride ion concentration in saliva in relation to dental caries has been done in the last fifty years. The only worker to report salivary chloride content in relation to dental caries was Hubbell[1] in 1933. Other workers who have reported the salivary chloride level, but not in relation to dental caries, were Clark and Shell,[2] and Clark and Levine.[3] Numerous studies of the effect of the chloride ion on the activity of the various substances isolated from saliva have been reported by such workers as Muus,[4] Rockwood,[5] and Schneyer.[6]

In this investigation, 251 children whose ages ranged from 3 years 6 months to 15 years 2 months were tested, and their dental conditions varied from complete absence of decay to mouths which exhibited the condition commonly referred to as "rampant decay." The various dental conditions encountered were subdivided into a number of categories, described below, in an endeavor to determine whether the present chloride level of the saliva had any association with the extent of dental caries encountered.

The evaluation of the dental data were based on the following classification:

A	Caries free	0 surfaces involved
B	Moderate dental decay	1-20 surfaces involved
C	Extensive dental decay	21 + surfaces involved

This is further subdivided as follows:

A	1 Caries free	
B	2 Moderate static decay	0 new cavities in last 12 months
	3 Moderate active slow decay	1-5 new cavities in last 12 months
	4 Moderate active rapid decay	6 + new cavities in last 12 months
C	5 Extensive static decay	0 new cavities in last 12 months
	6 Extensive active slow decay	1-5 new cavities in last 12 months
	7 Extensive active rapid decay	6 + new cavities in last 12 months

All the examinations and classifications in this study were done by one person. Drying of teeth and radiographs were used to increase accuracy of mirror and probe examination.

This investigation was supported in part by a Research Grant D-146-C from the National Institute of Dental Research of the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare.

Presented at the Thirty-third General Meeting of the International Association of Dental Research, Chicago, March 18-20, 1955. (J. D. Res. 34: 668, 1955.)

Received for publication April 11, 1955.

By the further subdivision of the DMF surfaces, it was hoped that a stage would be reached whereby it is possible to compare the present physiology of the saliva with the present oral condition in regard to tooth decay. The first part of the classification as presented above takes into account the over-all DMF condition, and the subdivisions are introduced to evaluate the present oral condition.

In dealing with subjects of the age range used in this study, 2 further difficulties enter into consideration: (a) A potential for decay, as suggested by salivary tests, may be present but not evident clinically because of an absence of a full complement of teeth on which these "cariogenic agents" can act. For this reason the period of exfoliation is a difficult one for conducting analytical studies of this type. (b) During the age period referred to above, the mechanical cleansing by saliva and food is far greater than if a full complement of teeth were present.

The subdivisions, as used here, may not do full justice to the results obtained, as the classification is best suited to a long-range caries prevalence study. Only about 65 per cent of the cases used in this investigation belong to a previous long-range study and could be classified strictly according to the above-described categories. A long-range follow-up of this investigation is planned.

Clark and Levine[3] reported a great variance in a comparison of resting and paraffin-stimulated saliva in regard to chloride, calcium, and phosphorus content. The results obtained in the present investigation are in agreement with these workers (Table I). Dreizen, Reed, Niedermeier, and Spies[7] likewise stated that variability is characteristic of saliva that is obtained after stimulation.

In a study on the inorganic constituents of saliva, Clark and Shell[2] have reported that a weekly testing of unstimulated saliva from 5 individuals over

TABLE I
RESULTS OBTAINED ON SALIVA FROM THE SAME INDIVIDUALS BEFORE AND AFTER STIMULATION BY CHEWING PARAFFIN

SUBJECT	RESTING WHOLE SALIVA (MEQ. CL/L.)	STIMULATED SALIVA (MEQ. CL/L.)
A	18.19	15.36
	17.20	15.29
B	20.47	35.36
	20.62	36.06
C	20.66	19.85
	18.38	18.38
D	24.64	22.82
	24.78	23.85
E	12.71	11.64
	13.25	12.71
F	16.42	15.78
	16.96	16.39
G	13.25	29.53
	13.57	
H	18.96	29.64
	18.91	30.84
I	17.21	31.39
	16.66	31.46

a 6-month period showed no appreciable alteration of chloride level, although the diet was changed 5 times during the 28 weeks of study. It is of some interest here to note that our own level of saliva was remarkably constant, even after ingestion of 1 to 2 Gm. of pure sodium chloride in gelatin capsules, and testing the saliva every half-hour for 6 hours after ingestion. No significant increase in chloride concentration was noted in 3 cases studied in this way (Table II).

TABLE II
EFFECT ON THE CHLORIDE LEVEL OF SALIVA AFTER INGESTION OF SODIUM CHLORIDE

TIME	SUBJECTS A (MEQ. CL/L.)	B (MEQ. CL/L.)	C (MEQ. CL/L.)
Preingestion	15.16	22.39	10.50
	15.74	22.33	11.64
30 minutes after ingestion	15.03	21.89	10.74
	16.14	19.25	11.00
60 minutes after ingestion	14.39	19.59	12.25
	15.30	19.39	11.57
90 minutes after ingestion	18.13	20.33	11.89
	16.08	20.47	12.10
120 minutes after ingestion	15.03	19.29	11.85
	15.46	19.02	12.10
360 minutes after ingestion	17.70	20.10	11.03
	16.75	19.62	11.03

Repeat tests of 4 individuals over a 4-month period showed a relatively constant chloride level (Table III).

TABLE III
REDUPLICABILITY OF CHLORIDE LEVEL IN SALIVA OF 4 INDIVIDUALS OVER A 4-MONTH PERIOD

DATE	SUBJECTS A (MEQ. CL/L.)	B (MEQ. CL/L.)	C (MEQ. CL/L.)	D (MEQ. CL/L.)
1/ 5/54	14.54	22.54	21.27	7.68
	15.54	24.00	21.45	7.72
1/11/54	9.88	20.03	22.04	
	12.91	19.98	19.73	
3/16/54	13.75	16.84	21.38	10.41
	14.03	19.34	21.59	10.45
3/25/54	15.16	22.39	16.89*	10.50
	15.74	22.33	16.89*	11.64
4/ 5/54	12.87	22.94		10.63
	12.42	24.66		11.19
4/ 8/54	18.19	20.66	20.47	
	17.20	18.38	20.62	
4/23/54		23.20		12.46
		24.21		12.67
4/27/54	18.93		20.15	
	19.01		20.15	
5/18/54	16.42	24.64		12.71
	16.96	24.78		13.25
Means	15.22	21.93	20.22	10.94

*Penicillin injection that day.

METHODOLOGY

Two methods previously used for blood serum chloride level determinations were tried and adapted for salivary work, those of Schales and Schales[8] and of Wilson and Ball.[9] Both methods were tried repeatedly and after sev-

eral adjustments conditions were developed that gave both satisfactory working conditions and reliable results. The results obtained by the use of each method were compared and contrasted. The use of the methods on a larger scale to test the chloride level of saliva appeared justified. Both methods gave almost identical results when used to test equal portions of a given sample of saliva and blood, and it was decided to employ the method of Schales and Schales in the larger-scale investigation as it was more readily adapted for work in the clinic.

SOLUTIONS USED

Mercuric nitrate, 0.26 Gm. (C. P. Baker's), is dissolved in water with the addition of 20 ml. 2 N nitric acid, and the volume is made up to 1,000 ml.

Indicator.—Diphenylcarbazone was used as the indicator, 50 mg. of it being dissolved in 50 ml. of 95 per cent alcohol. The solution was stored in a refrigerator, a fresh solution being prepared at least once a month.

Chloride Standard.—Sodium chloride was dried at 120° C., and 584.5 mg. was dissolved in water and made up to 1,000 ml. The solution contains 10 meq. chloride per liter and is used to standardize each batch of mercuric nitrate solution.

On testing whole saliva in the state in which it was collected, it was found that the chloride was almost undetectible, wherefore it was decided to deproteinize the saliva. This allowed a measurement of the chloride level. The following method was adopted for deproteinization:

To each 2 ml. of saliva were added: 1 ml. distilled water, 1 ml. 10 per cent sodium tungstate, and 1 ml. 2/3 N sulfuric acid.

This method of deproteinization was adapted from the method advocated by Folin and Wu[10] for blood serum. The deproteinized saliva was allowed to stand for 10 minutes and then filtered. The filtrate was used to evaluate the chloride level.

Determination.—To 1 ml. filtrate was added 8 drops of indicator, and the mercuric nitrate was then added from a microburette calibrated in 0.01 ml. intervals. The size of each drop is such that 1 ml. equals 100 drops. The clear, faintly pink filtrate turns an intense violet-blue on the addition of the first drop of mercuric nitrate in excess. The milliequivalents of chloride per liter of saliva were determined mathematically from the formula:

$$\frac{\text{Mercuric nitrate required}}{\text{End factor of standard solution}} \times \text{Dilution Factor} = \text{Meq. Cl/L. saliva}$$

RESULTS

Two hundred and fifty-one children were tested between January and June, 1954. The age of these children ranged from 3 years 6 months to 15 years 2 months. The range of the salivary chloride for this group varied from 6.49 to 37.88 meq. chloride per liter. All the tests reported in this investigation were done on saliva which had been obtained without mechanical stimulation. Fig. 1 represents the frequency distribution of the 251 cases investigated. Results are summarized in Table IV and presented graphically in Fig. 2.

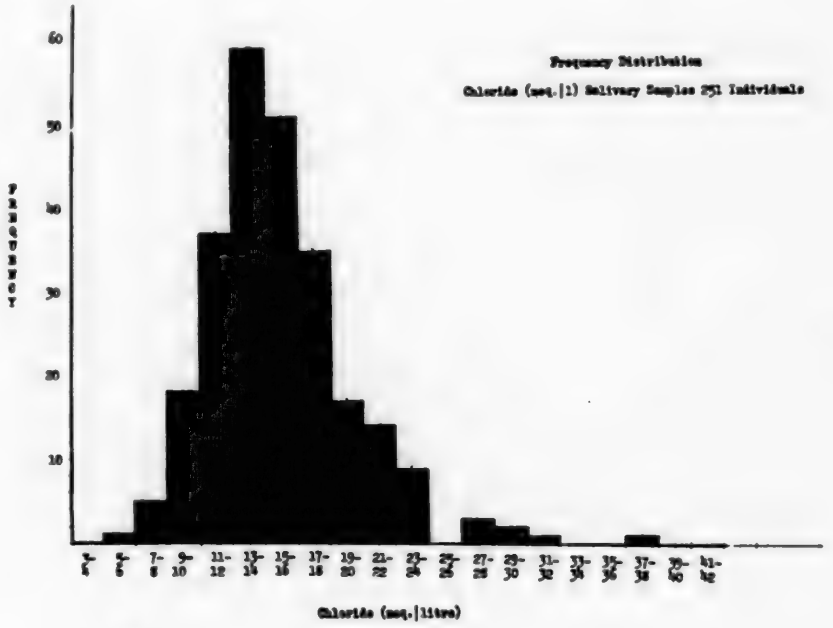

Frequency Distribution
Chloride (meq./l) Salivary Samples 251 Individuals
FREQUENCY
60
50
40
30
20
10
5- 6
6- 8
7- 9
9- 10
11- 12
13- 14
15- 16
17- 18
19- 20
21- 22
23- 24
25- 26
27- 28
29- 30
31- 32
33- 34
35- 36
37- 38
39- 40
41- 42
Chloride (meq./litre)

Fig. 1.

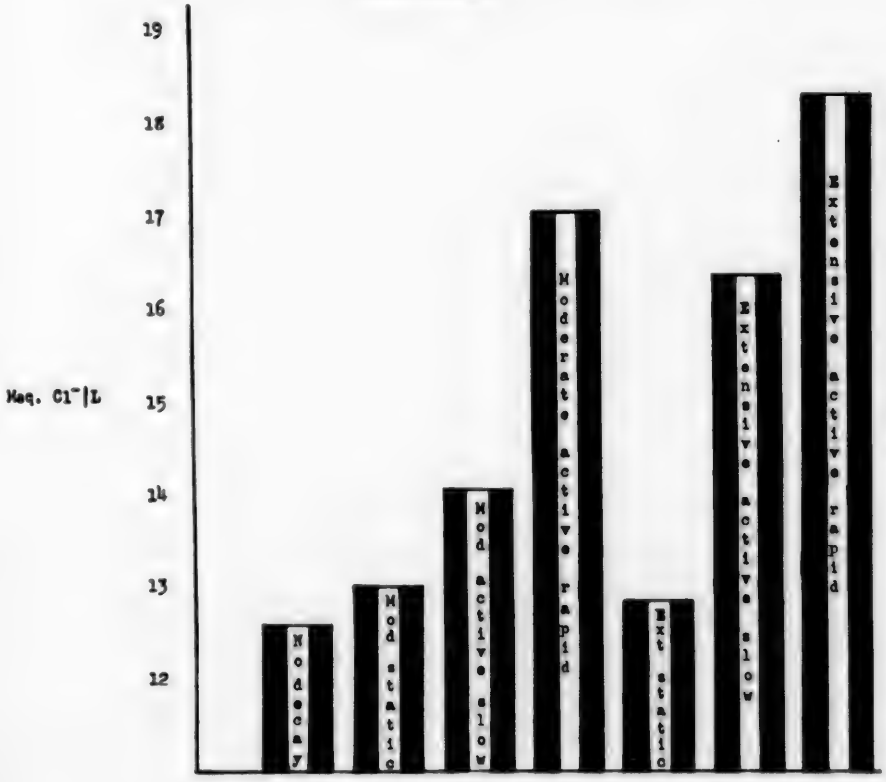

Meq. Cl⁻/L
19
18
17
16
15
14
13
12
Mod decay
Mod static
Mod active slow
Moderate active rapid
Ext static
Extensive active slow
Extensive active rapid

Fig. 2.

TABLE IV
THE MEAN, STANDARD DEVIATION, AND RANGE OF CHLORIDE VALUES FOUND IN THIS SERIES

GROUP DESIGNATION	DESCRIPTION	NO.	MEAN	ST. DEVN.	RANGE (MEQ. CL/L.)
0	Caries free	30	12.59	2.69	8.50-19.38
M-1	Moderate static decay	11	13.02	3.06	6.49-16.88
M-2	Moderate active slow decay	74	14.02	2.71	8.10-22.37
M-3	Moderate active rapid decay	41	17.12	3.70	11.36-29.93
E-1	Extensive static decay	2	12.83	---	---- ----
E-2	Extensive active slow decay	12	16.34	2.81	9.70-21.24
E-3	Extensive active rapid decay	81	18.35	4.84	11.35-37.88

TABLE V
GROUPS CONTRASTED USING "t" VALUES

Caries free	vs. 3 Moderate groups	"t" 3.46 0 vs. M-1, M-2, M-3
Caries free	vs. Ext. Act. Rapid group	"t" 6.10 0 vs. E-3
3 moderate groups	vs. Ext. Act. Rapid group	"t" 5.86 E-3 vs. M-1, M-2, M-3

Results shown in Table IV and V demonstrate that there exists an appreciable difference between the means of the 2 extreme groups, and that this difference is significant. The results of the other groups are such as to indicate that the present biologic status is related to the present dental condition. It will be interesting to see how this classification will hold up when more tests are made and the study becomes wholly longitudinal. Possibly the physiological state of saliva may prove prognostic of the coming dental condition.

Hubbell,[1] in her investigation of the association between the chloride ion level and caries experience, found no apparent correlation between the two. Her study, although carefully conducted, was with a small number of cases (32) only. In addition, her sampling technic may be questioned; some of the caries-free cases in her series had previous caries experience, and some cases developed caries before the conclusion of the experiment, as stated by the author.

DISCUSSION

According to these data there are significant "t" values between the incidence of caries and the chloride content of the saliva.

It may be that the role played by the chloride ion is an indirect one. The presence of the chloride ion appears essential for the satisfactory action of amylase on the ingested carbohydrates; its role seems to be that of either an activator, a catalyst, or a component of a coenzyme. Davidson,[11] Braun,[12] Sumner and Somers,[13] Rockwood,[5] Ernström,[14] Ege,[15] and Michealis[16] all have reported that it appears essential to have the presence of the chloride ion, in concentration of at least 0.02M, in order to facilitate the amylase activity. Schneyer[6] also found that the presence of the chloride ion activated amylase in the presence of inactivators like urease. Muus[4] reported that ". . . from chloride free solutions either the amylase did not precipitate, or the precipitate was amorphous. It is therefore suspected that the chloride ion forms an integral part of the amylase crystal."

Barmenkov[17] claimed that the chloride dissociates a mucin-amylase complex. Much more work will have to be done before one can gain any insight

into the mechanism of chloride action in the saliva. Although the nature of its action is not fully elucidated, in view of the above findings the importance of the chloride ion in the saliva has been established.

SUMMARY

A method for measuring the chloride ion concentration of the saliva and a method for classification of subjects according to the DMF index have been presented. On the basis of a study of 251 cases, a significant difference was found to exist between the chloride level of the caries-free group and that of the group with most extensive and rapid carious breakdown. The possible implications of the presence of the chloride ion in the saliva have been discussed, and it is suggested that there may be a connection between the presence of the ion and the amylase activity of the saliva.

REFERENCES

1. Hubbell, R. B.: The Chemical Composition of Saliva and Blood Serum of Children in Relation to Dental Caries, *Am. J. Physiol.* **105**: 436, 1933.
2. Clark, G. W., and Shell, J. S.: The Influence of Diet Upon the Inorganic Constituents of Human Saliva, *D. Cosmos* **69**: 500 and 605, 1927.
3. Clark, W. G., and Levine, L.: Inorganic Constituents of Human Saliva. II, *Am. J. Physiol.* **81**: 264, 1927.
4. Muus, J.: Studies on Salivary Amylase With Special Reference to Interaction With the Chloride Ions, *C. rend. Lab. Carlsberg* **28**: 10, 1953.
5. Rockwood, E. W.: The Effect of Neutral Salts Upon the Activity of Salivary Amylase, *Am. Chem. Soc. J.* **41**: 228, 1919.
6. Schneyer, L. H.: The Effects of Urea and Salts of Ammonium Phosphate on Salivary Amylase, *J. D. Res.* **31**: 767, 1952.
7. Dreizen, S., Reed, A. I., Niedermeier, W., and Spies, T. D.: Sodium and Potassium as Constituents of Human Salivary Buffers, *J. D. Res.* **32**: 497, 1953.
8. Schales, O., and Schales, S. S.: A Simple and Accurate Method for the Determination of Chloride in Biological Fluids, *J. Biol. Chem.* **140**: 879, 1941.
9. Wilson, W. D., and Ball, E. G.: A Study of the Estimation of Chloride in Blood and Serum, *J. Biol. Chem.* **79**: 221, 1928.
10. Folin, O., and Wu, H.: System of Blood Analysis: Preparation of Protein Free Blood Filtrates, *J. Biol. Chem.* **38**: 81, 1919.
11. Davidson, J.: *J. Agric. Res.* **70**: 175, 1945.
12. Braun, A. E.: *J. Biol. Chem.* **145**: 197, 1942.
13. Sumner, J. B., and Somers, G. F.: Chemistry and Methods of Enzymes, ed. 2, New York, 1947, Academic Press Inc.
14. Ernström, E.: Über den Temperaturkoeffizienten der Stärkespaltung und die Thermostabilität der Malzamylase und des Ptyalins, *Ztschr. phys. Chem.* **119**: 190, 1922.
15. Ege, R., and Oklitz, E.: Die Haltbarkeit des Ptyalins in verschiedenen Verdünnungen, *Biochem. Ztschr.* **219**: 422, 1930.
16. Michaelis, L., and Pechstein, H.: Die Wirkungsbedingungen der Speicheldiastase, *Biochem. Ztschr.* **59**: 77, 1914.
17. Barmenkov, Ya P.: Mechanism of Amylase Activation by Neutral Salts, *Biokhimyia* **4**: 160, 1939 via *Chem. Abstr.* **34**: 16938, 1940.

URINARY FLUORINE LEVELS IN CHILDREN FOLLOWING THE CLINICAL USE OF A STANNOUS FLUORIDE-CONTAINING DENTIFRICE

RICHARD A. SCHWEINSBERGER AND JOSEPH C. MUHLER

Indiana University, Department of Chemistry, Bloomington, Ind.

THE urinary fluoride level is considered to be a dependable measure of the fluoride intake, since the urinary fluoride output closely parallels the increase in fluorine intake that occurs when a fluoride deficient water supply is increased to 1.0 ppm.[1] However, a number of inorganic elements and dietary factors interfere with the storage of fluorine in the body.[2] Since a stannous fluoride-containing dentifrice has been shown to reduce the incidence of dental caries in children[3, 4] and adults[5] it is of considerable interest to determine what amount of fluorine is retained within the body through the unsupervised clinical use of this dentifrice. Since stannous fluoride in aqueous solution has been shown to cause the storage of less fluorine in the skeleton of the rat than comparable levels of sodium fluoride,[6] and since the stannous fluoride dentifrice contains an appreciable quantity of the elements known to interfere with fluoride storage,[2] it would seem that the use of such a product might contribute less fluorine to the skeleton than if an equal amount of fluorine were ingested from other sources.

The amount of fluorine in the urine was determined in a group of male children who resided in an area with a low concentration of fluorine in the water (F = 0.1 μg/ml.) and who used the stannous fluoride dentifrice for a minimum of 22 months. These data were compared with an identical group living in the same location and who used the same dentifrice but not containing stannous fluoride. Since it is generally accepted that children consuming water containing 1.0 ppm fluorine from birth have a significant reduction in the incidence of dental caries[7] as well as the absence of toxicity as judged by a long-term pediatric survey of children residing in such an area,[8] it was of interest to compare the urinary-fluoride dentifrice group with similar children who have used a communal water supply with a natural fluorine concentration of 0.85 ppm.

PROCEDURE

The two groups of children using the dentifrice* were obtained from the clinic at Indiana University, where the fluoride dentifrice was evaluated for its anticariogenic effect.[3] All of the children were residents of Bloomington, Indiana, where the communal water supply contains approximately 0.1 ppm

This work was supported in part by a grant from the Procter & Gamble Company, Ivorydale, Ohio.

Received for publication Feb. 18, 1955.

*The composition of the dentifrice used in this study was: heat-treated orthophosphate, 42 per cent; detergent (nonsoap), 2 per cent; humectant, 25 per cent; water, 29.2 per cent; binder, 1.4 per cent; stannous fluoride, 0.4 per cent; and flavor.

fluorine. The children who were used for comparative purposes lived in Ellettsville, Indiana, where the water supply naturally contained 0.85 ppm fluorine. All of the urine in both groups was collected for a 24-hour period from males only and was delivered to the clinic no later than the following day for chemical analysis.

The method of fluorine analysis was similar to that described by Smith and Gardner[9] and consisted of ashing duplicate 50.0 ml. aliquots of the urine sample with 1.0 Gm. of fluorine low calcium oxide* in a silicon crucible. Following thorough mixing of the urine—calcium oxide mixture, the sample was ashed at 600° C. for 6 hours. The entire contents of the crucible were then quantitatively transferred to the all-glass steam distillation unit and the fluorine distilled from perchloric acid at 137° C. The fluorine was titrated directly with thorium nitrate using alizarin red S as the indicator.

DATA AND DISCUSSION

The data from this experiment are seen in Table I and indicate the number of children in each group, their mean age, the mean volume of urine per person collected for each group, and the analytical fluorine data. A total of 211 children were used in this study with a mean age of 11 years for the group using the stannous fluoride dentifrice, 11.4 years for those using the control dentifrice, and 12.0 years for the group which were using the naturally fluoridated drinking water. The mean volume of urine as provided throughout a 24-hour collection period indicated that there was little difference in the total amount of urine excreted in the three groups. Even though approximately 100 ml. of urine less per person was collected in the group using the stannous fluoride dentifrice than that of the control dentifrice, this is not a sufficient quantity for concern, since the amount of fluorine in this volume would not affect these data or the conclusions drawn from them.

TABLE I

A COMPARISON OF THE URINARY FLUORINE LEVELS IN CHILDREN USING A STANNOUS FLUORIDE-CONTAINING DENTIFRICE AND A SIMILAR GROUP USING A NON-FLUORIDATED DENTIFRICE WITH CHILDREN USING A COMMUNAL WATER SUPPLY CONTAINING 0.85 PPM FLUORINE

	NUMBER OF CHILDREN	MEAN AGE (YEARS)	MEAN VOLUME (ML.)	F ANALYSIS	
				CONCENTRATION (μG/ML.)	TOTAL (MG.)
Stannous fluoride dentifrice	80	11.0	827	0.281	0.218
Control dentifrice	74	11.4	924	0.242	0.220
Fluorine water	57	12.0	852	1.010	0.841

The data obtained by analyzing the urine for fluorine indicate that the children who used the stannous fluoride dentifrice had only a minor increase in the urinary fluorine level. This is shown by an increase of only 14 per cent in fluorine concentration in the group using the fluoride dentifrice when compared to the group using the nonfluoride dentifrice. There are no differences in the total fluorine in the two dentifrice groups. These urinary values

*Special fluorine free (fluorine low) calcium oxide, obtained from Elmer and Amend, New York, N. Y.

correspond quite well with the data of McClure and Kinser obtained from children residing in an area with a similar amount of fluorine in the water supply.[10] These findings alone would suggest that little storage of fluorine in the skeleton is taking place. However, when the values obtained from the children who live in the natural fluoride area are compared with those using the stannous fluoride dentifrice, it is obvious that there is a much higher urinary fluoride level in the former group. The mean increase in the urinary level in children in the fluoride area is approximately 300 per cent for both fluorine concentration and total output when compared to the group using the stannous fluoride dentifrice. These data indicate that the use of this dentifrice containing stannous fluoride is not associated with any real increase in the storage of fluorine in the skeleton. Furthermore, the margin of toxicity appears well below that considered safe, since considerable evidence in the literature supports the view that children residing in a natural fluoride area in which approximately 1.0 ppm of fluorine is found in the water supply are without any toxic manifestations.[11]

CONCLUSIONS

The routine use of a stannous fluoride containing dentifrice (1,000 μg/Gm. of fluoride) does not significantly increase the urinary fluorine level when compared to a similar group of children using an identical dentifrice not containing fluoride. When the children using the stannous fluoride dentifrice are compared to a similar group of children residing in a natural fluoride area there is 300 per cent less fluorine in the urine in the former group.

REFERENCES

1. McClure, F. J.: Nondental Physiological Effects of Trace Quantities of Fluorine, page 77, in *Dental Caries and Fluoride*, F. R. Moulton, Editor, Amer. Assoc. Adv. Science, 1946.
2. Weddle, D. A., and Muhler, J. C.: The Effects of Inorganic Salts on Fluorine Storage in the Rat, *J. Nutrition* **54**: 437, 1954.
3. Muhler, J. C., Radike, A. W., Nebergall, W. H., and Day, H. G.: The Effect of a Stannous Fluoride-Containing Dentifrice on Caries Reduction in Children, *J. D. Res.* **33**: 609, 1954.
4. Muhler, J. C., Radike, A. W., Nebergall, W. H., and Day, H. G.: The Effect of a Stannous Fluoride-Containing Dentifrice on Dental Caries Reduction in Children. II. Caries Experience After One-Year, *J. A. D. A.* **50**: 163, 1955.
5. *Idem:* The Effect of a Stannous Fluoride-Containing Dentifrice on Dental Caries in Adults, *J. D. Res.* **35**: 49, 1956.
6. Muhler, J. C., Nebergall, W. H., and Day, H. G.: Studies on Stannous Fluoride and Other Fluorides in Relation to the Solubility of Enamel in Acid and the Prevention of Experimental Dental Caries, *J. D. Res.* **33**: 33, 1954.
7. Hatton, W. L., Linscott, B. W., and Williams, D. B.: Brantford, Ontario, Fluoridation Study, *J. A. D. A.* **49**: 183, 1954.
8. Schlesinger, E. R., Overton, D. E., and Chase, H. C.: A Long-Term Medical Study of Children in a Community with a Fluoridated Water Supply, pp. 131-147, in *Fluoridation as a Public Health Measure*, J. H. Shaw, Editor, Amer. Assoc. Adv. Science, 1954.
9. Smith, F. A., and Gardner, D. E.: Fluoride Studies, U.S.A.F.C., Official Bulletin, A.E.C.D.-2161.
10. McClure, F. J., and Kinser, C. A.: Fluoride Domestic Waters and Systemic Effects. II. Fluorine Content of Urine in Relation to Fluorine in Drinking Water, *Pub. Health Rep.* **59**: 1575, 1944.
11. Hodge, H. C., and Smith, F. A.: Some Public Health Aspects of Water Fluoridation, pp. 79-109, in *Fluoridation as a Public Health Measure*, J. H. Shaw, Editor, Amer. Assoc. Adv. Science, 1954.

EFFECT OF VARIOUS CONCENTRATIONS OF SODIUM FLUORIDE ON PARASITIC AND FREE-LIVING PROTOZOA AND ROTIFERA

WAYNE W. WANTLAND

Department of Biology, Illinois Wesleyan University, Bloomington, Ill.

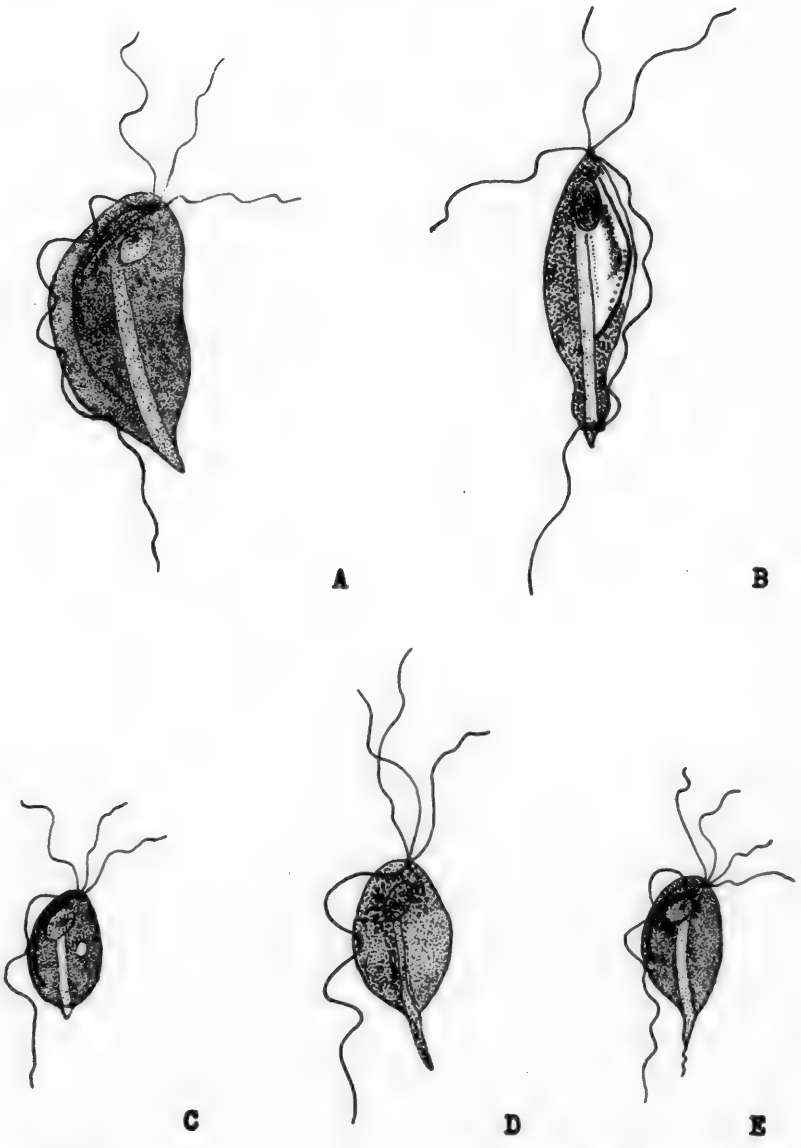
PRELIMINARY studies in this laboratory on the toxicity of sodium fluoride in 24 hamsters have indicated that the LD50 (dosage from which 50 per cent of the animals die) is somewhat higher for the hamster (70 to 80 mg. per kilogram of body weight) than that reported for the rat (50 mg. per kilogram of body weight) by Cox and Hodge.[1] Autopsy of 50 animals that had been given from 2 to 10,000 ppm NaF daily in their drinking water for three weeks and examination of 20 hamsters that had received 0.5 c.c. of 100 to 10,000 ppm daily for five days by stomach tube revealed diminished numbers of trichomonads, parasitic flagellated protozoa almost always found in the cecum of these rodents (Fig. 1). The stronger fluoride solutions effected the greatest decrease in numbers and the higher concentrations (500 to 10,000 ppm) resulted in approximately the same degree of loss of these parasites as the administration of carbarsone and the feeding of a high protein diet to infected hamsters reported by Wantland and Johansen.[2]

This in vivo finding suggested investigation of the in vitro behavior of trichomonads from the hamster in various concentrations of NaF. These parasitic protozoa exhibit a definitely different reaction to different concentrations of NaF. Fairly concentrated numbers of these flagellates were obtained by sacrificing an animal, excising a portion of the cecum 1 cm. long, measuring from the blind end, and placing it in 3 c.c. of 0.85 per cent NaCl. Subjecting 1 drop of this culture to 2 drops of 500 to 10,000 ppm NaF on a microscope slide for 40 minutes resulted in inactivation, some cyst formation (Figs. 2 and 3), and negative reactivation upon the addition of 1 drop of 0.85 per cent NaCl. Subjecting 1 drop of culture to 2 drops of 2 to 100 ppm NaF and control distilled water on a slide for 40 minutes resulted in a slowing of movement and formation of cysts in all preparations but a decided increase in reactivation progressing from the 100 ppm preparations to the 2 ppm preparations with almost complete reactivation in the 2 ppm and in control water preparations upon the addition of 1 drop of 0.85 per cent NaCl (Table I).

The behavior pattern manifested by these parasitic protozoa when subjected to various concentrations of NaF suggested further study using free-

This study was supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army under Contract No. DA-49-007-MD-495.

Received for publication March 25, 1955.



Figs. 1, *A-E*, 2, 3, 4, 5, 6, 10, 11, and 12 are photographs of camera-lucida drawings.

Fig. 1.—*A*, *Trichomonas muris* (×3,000). *B*, *Trichomonas cricetus* (×3,000). Note slender shape and posterior bulbar expansion. *C*, *Trichomonas minuta* (×3,000). *D*, *Trichomonas wenyoni* (×3,000). Note long protrusion of axostyle at posterior end. *E*, *Trichomonas microti* (×3,000). Note 4 anterior flagella.

living forms. The behavior patterns of Paramecia (Fig. 4), Euglena (Fig. 5), and Rotifera (Fig. 6) in various concentrations of NaF are shown in Figs. 7, 8, and 9. In all cases, 0.6 c.c. (10 drops) of culture was added to 3 c.c. (50 drops) of the various NaF concentrations.* The species of Paramecia and Euglena show similar patterns of resistance. When placed in higher concentrations (1,700 ppm to 10,000 ppm), these organisms are killed rather

Fig. 2.

Fig. 3.

Fig. 2.—Precystic stage of Trichomonad.
Fig. 3.—Cystic stage of Trichomonad.

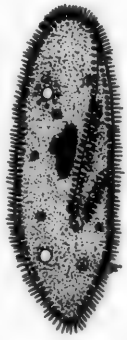


Fig. 4.

Fig. 5.

Fig. 6.

Fig. 4.—*Paramecium caudatum* (×200).
Fig. 5.—Semidiagrammatic drawing of Euglena (×1,300).
Fig. 6.—Rotifera—*Philodina roseola* (×200).

quickly. They live for 7 hours when culture is placed in 1,500 ppm, 8 days in 1,400 ppm, and 15 days in 1,250 ppm. When placed in concentrations of 2 ppm to 1,000 ppm and in distilled water controls, they continue to live, reproduce, and show a degree of activity comparable to that found in original

*Since the amounts of culture water used contained no measurable amount of sodium or fluorine, the actual concentrations (ppm NaF) in which the behavior of organisms was observed were five-sixths (83.3 per cent) of the concentrations shown in ppm in Figs. 8, 9, and 10.

cultures. The pattern of resistance manifested by the Rotifera, while somewhat similar to that of Paramecia and Euglena, shows considerable variation (4 or 5 hours to 9 days) in the ability of individual organisms to survive

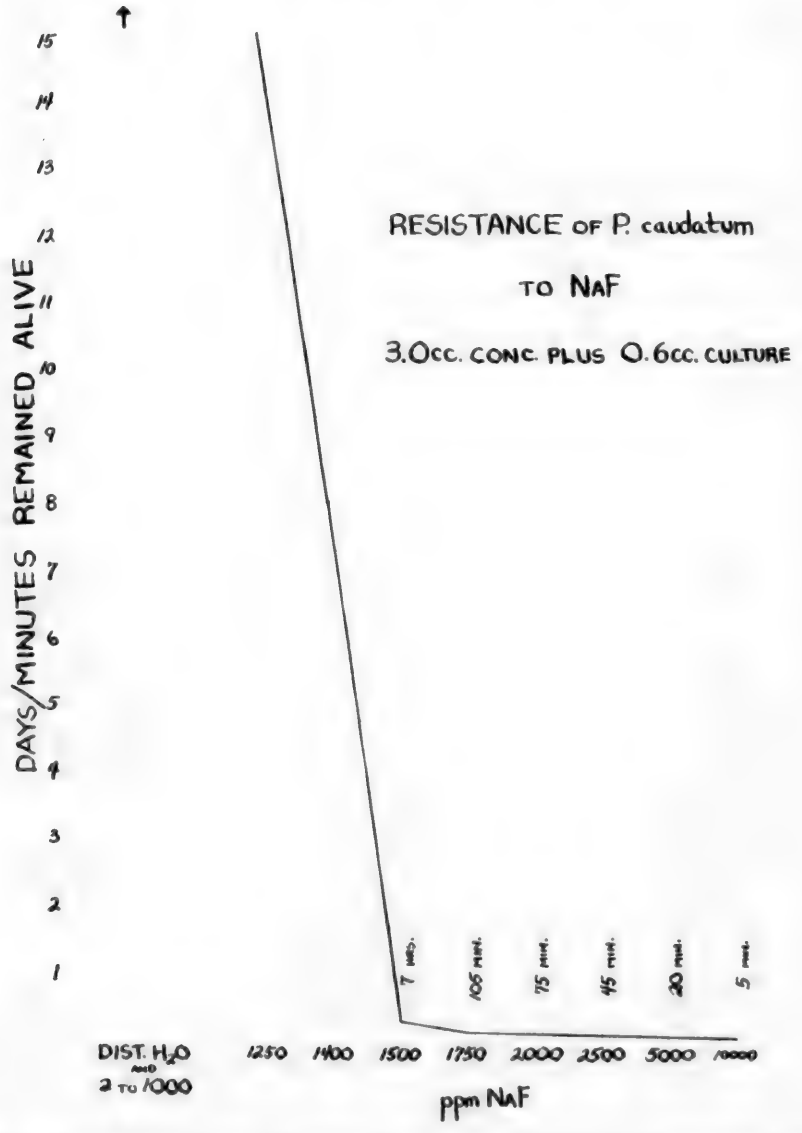


Fig. 7.—Graph showing effect of various concentrations of NaF on *Paramecium caudatum*.

when placed in 1,250 ppm to 1,450 ppm NaF. As in the case of the Protozoa, the Rotifera continue to live, reproduce, and are very active when placed in

concentrations of 2 ppm to 1,000 ppm. That the toxic effect upon these organisms in the higher concentrations of NaF was due to the F ion was indicated by controls in which they all remained alive for as long as 10 days in 2,125 ppm to 4,250 ppm NaCl.

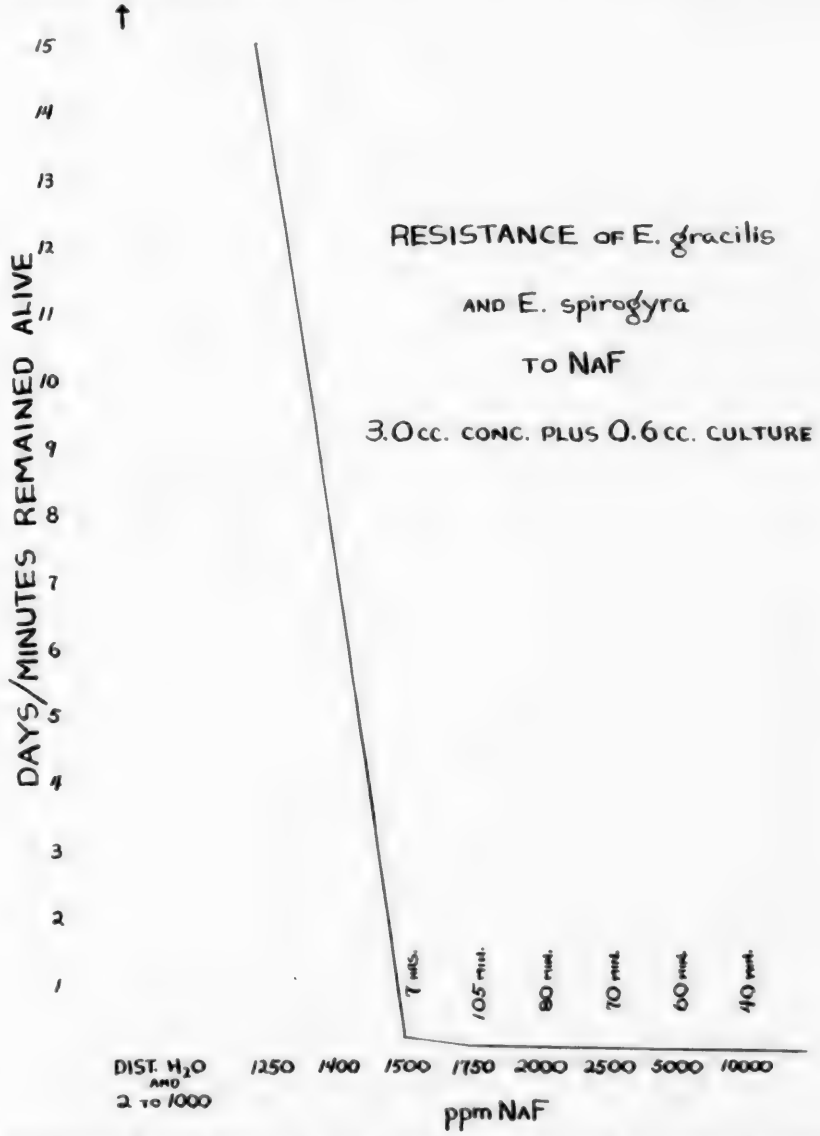


Fig. 8.—Graph showing effect of various concentrations of NaF on *Euglena gracilis* and *Euglena spirogyra*.

The ability shown by these free-living organisms to live in lower concentrations of NaF and the rather marked difference shown in periods of survival when placed in 1,250 ppm, 1,400 ppm, and 1,500 ppm, as opposed to

their susceptibility to 1,750 ppm and higher concentrations, led to the consideration of and search for a devitalizing agent which would weaken test organisms just enough to permit changes in behavior pattern which in turn would indicate as precisely as possible the relative strength of lower concentrations (2 ppm to 1,000 ppm NaF) in which the organisms were placed.

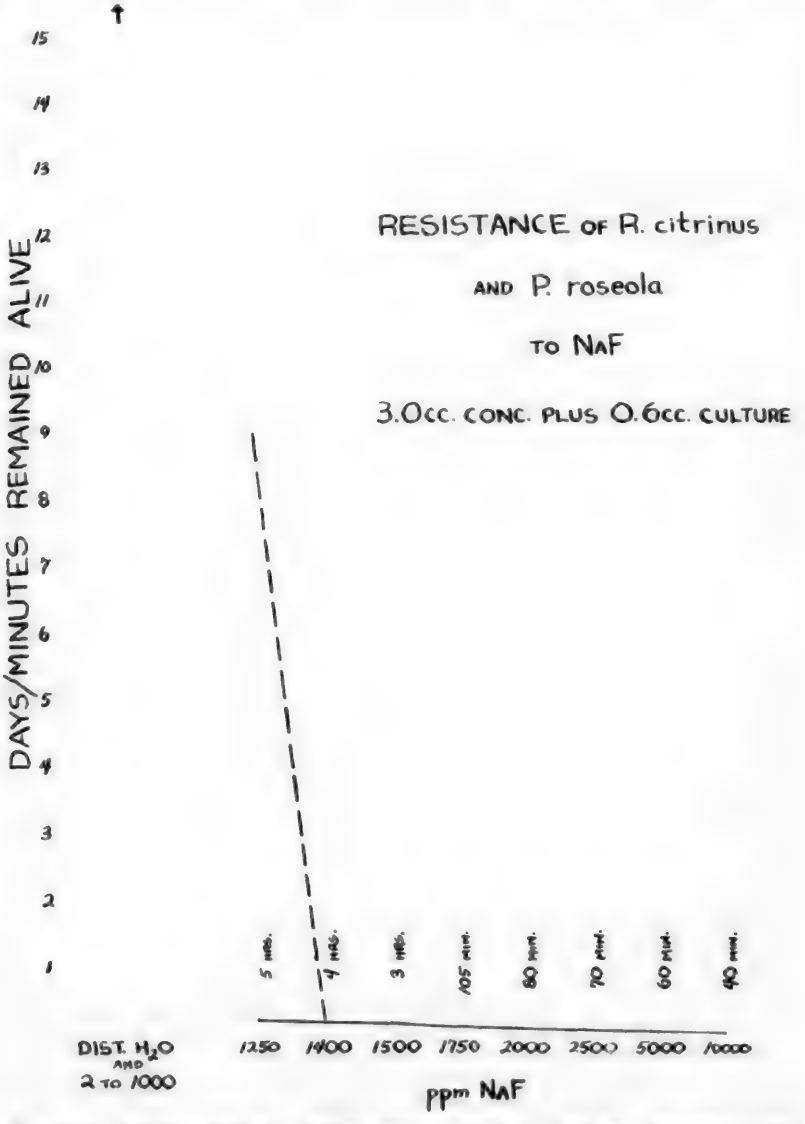


Fig. 9.—Graph showing effect of various concentrations of NaF on *Philodina roseola* and *Rotifera citrinus*.

After many trials using dilutions of acids, ethyl alcohol, and other fixatives, a modification of the Hellige Fluoride Reagent No. 611-S751 was selected

TABLE I

EFFECT OF NaF CONCENTRATIONS (2-10,000 PPM) ON TRICHOMONADS FROM HAMSTER

CONCENTRATIONS	INACTIVATION	REACTIVATION (NaCl) AFTER 40 MINUTES
Distilled H_2O	Movement slowed (cyst formation)	Almost complete
Bloomington tap H_2O	Movement slowed (cyst formation)	Almost complete
2-50 ppm NaF	Movement slowed (cyst formation)	Almost complete
100-300 ppm NaF	Movement slowed (cyst formation)	Occasional
500-2,000 ppm NaF	Movement slowed (cyst formation)	Negative
2,500-10,000 ppm NaF	Trophozoites killed (very few cysts formed)	Negative

as an efficient devitalizing agent. Rotifera were chosen to work with because of the precise changes observed in behavior patterns and the ease of culturing. The modification of the Hellige Reagent (MHR) consists of the following: 0.336 per cent hydrochloric acid (HCl); 0.111 per cent sulfuric acid (H_2SO_4); and 0.001 per cent zirconium oxychloride ($ZrOCl_2 \cdot 8H_2O$) pH 1.3 to 1.4. Using Rotifera as test forms and MHR as a devitalizing agent, a biological test was devised which has accurately differentiated between water containing 10 ppm or less NaF, and water containing 20 or more ppm NaF. To date, distilled water and drinking water from 20 communities in central and north central Illinois* (range of pH 5.0 to 8.0) have been successfully subjected to this test NaF added to H_2O in laboratory).

The following test was made of each of these waters: (1) Ten 10 c.c. vials were thoroughly cleaned and finally rinsed with the water to be tested. (2) Into each of 2 vials (controls) were placed 0.6 c.c. (10 drops) of the sample of water to be tested. (3) Into each of the 8 remaining vials were placed 0.6 c.c. of the sample water containing the following ppm of NaF: 2 – 10 – 20 – 50 – 100 – 500 – 1,000 – 5,000. (4) MHR was added to each vial except 1 control in an amount dependent upon the pH of the sample water. For waters with a pH of 7.0 or below, 1 drop of MHR was used and for waters with a pH of 7.2 or above, 2 drops of MHR were added. (5) To each vial was added 0.6 c.c. of Rotifera-Paramecia culture maintained at a pH of 6.8 to 7.0. Paramecia as indicator organisms act as a further check of the proper pH for the test. If the correct amount of MHR (1 or 2 drops) has been added, the Paramecia will be killed displaying trichocysts in from 5 to 10 minutes following addition of the culture (Fig. 10). After 2 hours, the contents of each vial was examined microscopically by placing 4 or 5 drops on a clean slide without cover glass and observing under low power. The control vial without MHR revealed living, active Rotifera and Paramecia. The control to which

*Bloomington, Normal, Ottawa, Sheridan, Somonauk, Leland, Minonk, Springfield, Urbana, El Paso, Streator, Lincoln, Wedron, Serena, Earlville, LeRoy, Danvers, Decatur, Pontiac, Marseilles.

MHR had been added contain dead Paramecia and retracted Rotifera. Occasionally, Rotifera were observed in both controls which had retracted into round shapes by telescopic action of segments (Fig. 11). Examination under high power after placing a cover glass on the preparation revealed that these retracted forms of Rotifera were definitely alive as manifested by twisting and churning movements of parts of the body and contraction and relaxation of the mastax. Examination of the contents of vials containing 2 ppm and 10 ppm NaF revealed dead Paramecia and retracted, live Rotifera with an occasional dead, extended form of the latter organism. All remaining vials (20 – 50 – 100 – 500 – 1,000 – 5,000 ppm NaF) contained dead Paramecia and only dead, extended Rotifera showing extensive plasmolysis (Fig. 12).

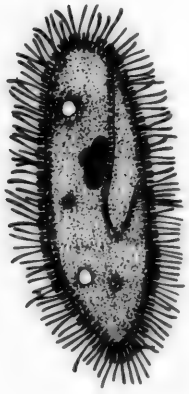


Fig. 10.

Fig. 11.

Fig. 12.

Fig. 10.—*Paramecium caudatum* showing expelled trichocysts.
Fig. 11.—Rotifera retracted by telescopic action.
Fig. 12.—Dead, extended Rotifera showing extensive plasmolysis.

Such a test as the one just described, using Rotifera as test organisms in determining amounts of fluorine in water in excess of 9 or 10 ppm, might be of value in supplementing the usual colorimetric procedure. Both Rotifera and Paramecia are easily maintained in cultures. In practice, the test would be relatively simple and could be completed in 2 hours as follows: (1) Determine pH of sample of water with pHydrion papers. (2) Place 0.6 c.c. (10 drops) of the sample water in a clean 10 c.c. vial. (3) Add MHR (1 or 2 drops as indicated by pH of sample water). (4) Add 0.6 c.c. (10 drops) of Rotifera-Paramecia culture. (5) If only 1 drop of MHR was used, examine in 10 minutes and add 1 more drop of MHR if Paramecia are alive and active. Examine again at the end of 2 hours. If the sample water contained 20 ppm NaF (9 ppm F) or more, all Paramecia will be dead, displaying trichocysts, and all Rotifera will be dead and extended. If the sample water contained 10 or less ppm NaF, all Paramecia will be dead but living Rotifera will be

noted, most of them in contracted, rounded form and perhaps a few exhibiting active locomotion. Two more vials (controls) could easily be added in practice, both containing 0.6 c.c. of the sample water before fluorides were added, one of them receiving MHR and both receiving 0.6 c.c. of culture.

The explanation of the physiologic reactions manifested by Rotifera in this test is not clear. It is possible that the devitalizing agent MHR acts as a trigger mechanism which sets off physical and chemical changes in the protoplasm of the organisms, particularly the membranes of cells, resulting in increased permeability which would then permit faster and more potent action of the F ion. There are certainly 2 toxicity factors involved (MHR and F ion), as shown by experiments cited earlier in this paper in which both Paramecia and Rotifera survived for weeks in 2 to 1,000 ppm NaF, and the toxicity of higher concentrations of NaF was demonstrated to be due to fluorine rather than sodium by controls in which the organisms remained alive for as long as 10 days in 4,250 ppm NaCl.

Certainly more waters need to be subjected to this test before unquestioned reliability can be established. A complete analysis of waters tested experimentally would seem advisable in order to control any samples containing substances or exhibiting characteristics which might conceivably affect the test. The results of experiments to date indicate that pH can be controlled and that hardness (Ca and Mg salts) and small amounts of sulphur and iron as well as ordinary chlorination do not present problems.

It is suggested that the data presented in these studies on resistance of Protozoa and Rotifera to sodium fluoride may be employed as a further aid in combating the idea prevalent in many areas that fluorine is a highly toxic protoplasmic poison even in low concentrations. It is the hope of the writer that the results of the biological test for fluorine, using Rotifera as test organisms, will further promote testing procedures for confirming amounts of fluorine added to waters which should markedly allay the fears of the most critical and apprehensive individuals.

SUMMARY

1. The LD50 (dosage from which 50 per cent of the animals die) of NaF for hamsters was found to be of the order of 70 to 80 mg. per kilogram of body weight.

2. Marked decrease in numbers of trichomonads (parasitic flagellated Protozoa) was observed in the cecum of hamsters following administration of 2 to 10,000 ppm NaF.

3. In vitro experiments in which trichomonads from the hamster were subjected to various concentrations of NaF (2 to 10,000 ppm) revealed specifically different behavior patterns in controls (distilled water) and 2 to 100 ppm NaF as opposed to 500 to 10,000 ppm NaF.

4. In vitro experiments in which free-living forms of Protozoa and Rotifera were subjected to various concentrations of NaF revealed the remarkable ability of these organisms to survive and reproduce when placed in

concentrations of 2 to 1,000 ppm NaF. When placed in concentrations of 1,700 to 10,000 ppm NaF, these organisms were quickly killed.

5. A biological test is described in which Rotifera were employed as test organisms in differentiating between waters containing 10 or less ppm NaF and waters containing 20 or more ppm NaF.

REFERENCES

1. Cox, G. J., and Hodge, H. C.: The Toxicity of Fluorides in Relation to Their Use in Dentistry, *J. A. D. A.* **40**: 440, 1950.
2. Wantland, W. W., and Johansen, E.: Effect of Carbarsone, Chiniofon and High Protein Diet on Trichomonads in the Intestine of the Golden Hamster, *J. Parasitol.* **40**: 479, 1954.

X-RAY STUDY OF FLUORAPATITE FORMATION DURING THE FLUORIDE TREATMENT OF POWDERED DENTAL ENAMEL

ROBERT B. FISCHER, PH.D., CHARLES E. RING, PH.D., AND JOSEPH C. MUHLER, D.D.S., PH.D.

Department of Chemistry, Indiana University, Bloomington, Ind.

IT HAS been reported that treatment of dental enamel with fluoride reagents results in a change of the initial apatite structure to a structure of calcium fluoride.[1-3] This change begins on the surface of the enamel with the rate of change increasing as the pH of the treating solution is lowered. Not all fluoride reagents produce this change. These conclusions have been based on x-ray diffraction and electron diffraction data from which it was not possible to distinguish between hydroxyapatite and fluorapatite. The apatite of dental enamel is largely hydroxyapatite. Therefore, the question remained as to whether fluorapatite could be either a second product or a stable intermediate in the treatment of dental enamel with fluoride reagents. Other workers have investigated this problem on the basis of chemical analysis and have obtained useful information.[4] However, there is no direct chemical test for fluorapatite in the presence of hydroxyapatite and calcium fluoride.

In this laboratory, two types of experimental work have been undertaken to assay further the effects of the fluoride treatments upon the structure of dental enamel. One of these is infrared spectrophotometry.[5] The present paper describes further x-ray diffraction experiments. Two methods of x-ray diffraction work have been used. One is a conventional powder diffraction method with particular attention to geometrical factors so as to provide optimum resolution. In the other, a curved crystal monochromator and camera have been designed, constructed, and used. Photographic recording of the patterns was used throughout. It was hoped that the resolution of one or both methods would be adequate to permit distinguishing between hydroxyapatite and fluorapatite.

EXPERIMENTAL METHODS AND OBSERVATIONS

Method One.—

Any one or more of several factors may limit the line sharpness and, accordingly, the resolution of a conventional cylindrical x-ray powder diffraction pattern. Some of these factors may be tabulated as follows.

A. Factors due to the specimen itself:

1. Particle size.
2. Its crystallographic purity.

Presented at the Thirty-third General Meeting of The International Association for Dental Research, Chicago, March 18-20, 1955 (*J. D. Res.* 34: 685, 1955).

Received for publication April 20, 1955.

B. Factor due to the mounting of the specimen:

1. How closely it approximates a point (or line) at the center of the camera.

C. Factors due to the x-radiation used:

1. How strictly monochromatic it is.
2. How well collimated it is.
3. How fine a pencil of rays is used, i.e., how small the limiting pinholes are.

D. Factor due to the film:

1. Its grain size, or its resolution.

The A factors are largely beyond the control of the analyst. Dental enamel is not a single, well-crystallized substance. It is a composite of several substances, both inorganic and organic, and doubtless includes some solid solution components. Therefore, these factors set an ultimate limit upon resolution beyond which no x-ray diffraction technic can go.

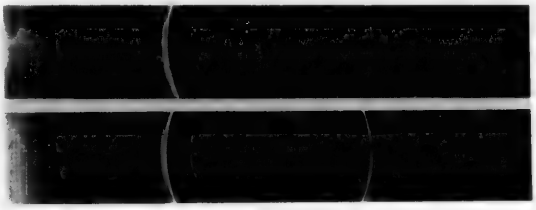


Fig. 1.—X-ray diffraction patterns of dental enamel (hydroxyapatite) (top) and of synthetic fluorapatite (bottom) as taken by refined conventional method.

The B factor was found to be of some significance in this study. The powdered specimen is usually mounted on or in a thin glass or plastic tube. The diameter of this tube markedly influences the sharpness of the resultant patterns, even with a standard camera of radius 114.59 mm. Tubes with diameters as large as 0.25 mm. are commonly used. In this study, a diameter of slightly less than 0.1 mm. was found to be about a practical minimum.

The C 1 factor was emphasized in method two, but a conventional nickel filter was used in method one. Collimation of the x-radiation is conventionally accomplished with a series of pinholes or slits, typical limiting diameter being within the range of 0.005 to 0.040 inch. As the size is decreased, the resolution is improved, but the necessary exposure time is increased. In this study, a pinhole size of about 0.003 or 0.004 inches was used, and the resulting large exposure times were accepted as inevitable.

The D factor is not of significance in comparison to the others listed.

With these experimental considerations of the B, C, and D factors, it is felt that the resulting line breadths are limited largely by the A factors.

Patterns of hydroxyapatite (dental enamel) and fluorapatite are shown in Fig. 1. These patterns are very similar, even upon close measurement. The crystallographic structures of the 2 apatites are almost identical.

The first and the fourth dominant rings away from the center are, respectively, the second and the first most intense lines. Measurements of ring diameters for both of these lines in patterns are listed in Table I. The listed values are averages taken of measurements on several patterns of several samples of each type of apatite. The individual measurements of ring diameter were usually within about 0.1 millimeter.

TABLE I

RING DIAMETERS FOR DIFFRACTION LINES OF FLUORAPATITE AND HYDROXYAPATITE

	FLUORAPATITE	HYDROXYAPATITE
Fourth ring from center		
diameter	63.8 mm.	63.5 mm.
d value (Å)	2.797	2.812
First ring from center		
diameter	51.7 mm.	51.8 mm.
d value (Å)	3.446	3.444

The observed variation of measurements on replicate patterns is such that identification of one apatite or the other directly by means of measured ring diameter is not possible. Film shrinkage measurements revealed values ranging from 0.0 to 0.8 mm. in 180.0 mm., with most values being with 0.3 mm. in 180.0 mm. So identification of a ring diameter within less than 0.1 or 0.2 mm. in about 60.0 mm. is likely unreasonable. However, a measurement of the distance, on either side of the center spot, between these 2 rings is useful. This measurement may be made conveniently with a magnifying, internally calibrated, Bausch and Lomb eyepiece. This measured value repeatedly is found to be 6.1 mm. for fluorapatite and 5.8 mm. for hydroxyapatite. Both values are reproducible within 0.1 mm. from one pattern to another of the same apatite. Only a small portion of the film is used for each measurement, so film shrinkage and related factors are of little or no consequence.

It is now felt that this measurement does provide a valid means of distinguishing between hydroxyapatite and fluorapatite. Small amounts of one in the presence of the other cannot be identified, but one or the other can be identified as the major constituent.

Method Two.—

In this series of experiments, the resolution of the x-ray diffraction patterns was improved by means of a more effective monochromator, factor C 1 in the listing above. The conventional procedures use a metal or metal oxide foil as a filter. Such a monochromator is adequate for most purposes but the radiation passing through is not strictly monochromatic. An experimental apparatus has been designed, constructed, and used employing a curved crystal as the monochromator in place of the foil filter. The design is similar to that described by Guinier,[6] and the unit constructed and used in this laboratory is described in detail elsewhere.[7] Therefore, only an abbreviated description will be given here. Radiation from a conventional x-ray tube strikes a properly ground and curved quartz or mica crystal. The crystal is so aligned that the Ka radiation is diffracted into the camera in a self-focusing arrangement. The specimen is placed at the entrance to the camera proper. Photographic recording of the patterns is used.

A typical pattern of an apatite is shown in Fig. 2. The lines are very sharp near the center portion, so resolution is good. In addition, the background intensity is very light, relative to line intensity, so the sensitivity of the method is better than in more conventional technics.

In Fig. 3 are shown enlarged views of portions of the patterns of fluorapatite and of hydroxyapatite (dental enamel), both samples containing sodium chloride as an internal standard. The most dominant line of sodium chloride

Fig. 2.—X-ray diffraction pattern of synthetic fluorapatite as taken by curved crystal monochromator technic.

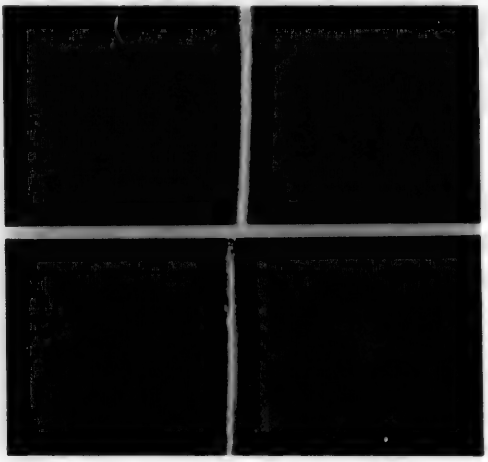


Fig. 3.—Enlarged views of parts of patterns of NaCl + dental enamel (top) and of NaCl + fluorapatite (bottom) as taken by curved crystal monochromator technic.

has a d value of 2.81 Å. This line overlaps the d = 2.81 line of hydroxyapatite, but is resolvable from the d = 2.80 line of fluorapatite. Thus a means is provided of distinguishing between these two apatites. This distinction between the two may alternatively be based upon the measured distance from the d = 3.44 line to the d = 2.81/2.80 line, as was done in method one. No added internal standard substance is required in this case. Once again, it is not possible to identify small amounts of one apatite in the presence of the other, but one or the other may be identified as the major constituent.

APPLICATION TO TREATED ENAMELS

Several samples of powdered dental enamel were treated with various fluoride reagents as was done in earlier work.[1, 2] These treated samples were

examined both by method one and by method two to see whether the fluorapatite structure appeared. In no case was the fluorapatite structure found. In all cases, the apatite structure persisted as that of hydroxyapatite, even when the treatment was so severe that calcium fluoride was formed and became the dominant component.

Therefore, it can be concluded that fluorapatite is neither a major second product nor a major stable intermediate in the change of hydroxyapatite to calcium fluoride upon treatment of powdered dental enamel with various fluoride reagents.

This is helpful information with respect to the protection of teeth by topical application of fluorides. It would be of interest to ascertain whether or not fluorapatite forms from systemic ingestion of fluorides. It would be of further interest to ascertain whether fluorapatite forms in lesser amounts which would not be detected by these x-ray methods. The infrared analysis[5] may provide such information.

SUMMARY

Two x-ray methods are described for the direct determination of whether an apatite sample is dominantly hydroxyapatite or dominantly fluorapatite. In one, conventional powder diffraction technics are used with particular attention to means of collimation and of sample mounting to insure optimum resolution. By measuring the distance from the line at d = 2.81/2.80 Å to the line at d = 3.44 Å, it is possible to distinguish between the two apatites. In the other method, a curved crystal monochromator is used to insure sharp lines, and sodium chloride is used as an internal standard. Again, the two apatites may be distinguished from each other. Samples of dental enamel treated with fluoride reagents reveal a persistence of the hydroxyapatite structure even when treatment is so severe that much calcium fluoride is formed. It is concluded that, under the conditions used, fluorapatite is not the dominant product in the treatment of enamel with fluorides.

The authors wish to acknowledge the technical assistance of Robert H. Duff. This research was supported in part by the Medical Research and Development Board of the Office of the Surgeon General, Department of Army, under Contract No. DA-49-007-MD-238.

REFERENCES

1. Fischer, R. B., and Muhler, J. C.: The Effect of Sodium Fluoride Upon the Surface Structure of Powdered Dental Enamel, *J. D. Res.* **31**: 751, 1952.
2. Fischer, R. B., Muhler, J. C., and Wust, C. J.: The Effects of Several Fluoride Reagents Upon the Surface Structure of Powdered Dental Enamel, *J. D. Res.* **33**: 50, 1954.
3. Fischer, R. B., Elsheimer, H. N., and Muhler, J. C.: The Effects of Several Fluoride Reagents on the Structure of Whole Teeth, *J. D. Res.* **33**: 538, 1954.
4. McCann, H. S., and Bullock, F. A.: Reactions of Fluoride Ion With Powdered Enamel and Dentin, *J. D. Res.* **34**: 59, 1954.
5. Ring, C. E., Fischer, R. B., and Muhler, J. C.: An Infrared Study of Dental Enamel and Related Substances, *J. D. Res.* **34**: 685, 1955.
6. Guinier, A.: X-Ray Crystallographic Technology, English Translation by T. L. Tippell, London, 1952, Hilger and Watts, Ltd., Ch. 6.
7. Ring, C. E.: Some Physical and Chemical Methods in the Study of I. Electrodeposited Nickel and II. The Dental Enamel—Fluorapatite System, Ph.D. Thesis, Indiana University, 1955.

THE FORMATION OF LACTIC ACID IN DENTAL PLAQUES

I. Caries-Active Individuals

B. W. MOORE,* WM. J. CARTER, J. K. DUNN, AND L. S. FOSDICK

(Northwestern University Dental School, Chicago, Ill., and U. S. Naval Training Center, Great Lakes, Ill.)

MUCH evidence, more or less indirect, supports the theory that a least one of the causative factors in dental caries is a local increase in hydrogen ion concentration due to the metabolism of carbohydrates by bacteria in the mouth. Salivary pH, itself, does not fall appreciably in the mouth after eating carbohydrates[1-3]; however, the pH does reach low values in the plaque found on the surface of teeth.[4-10] These findings seem to support earlier suggestions that plaque is the site of formation of acids from sugars and of the effect of hydrogen ion on teeth. The composition and mechanism of formation of plaque have not yet been investigated in detail. Therefore, for the purpose of this work, the plaque is defined in an operational manner as any material, whatever its composition or origin, that can be removed readily from the surface of tooth by means of a dental instrument.

Some evidence is available concerning the possible identities of the acids which accumulate in plaque. Lactic acid is produced in vitro in the presence of plaque material, presumably by anaerobic metabolism.[11] Other acids formed in vitro (acetic, formic, and pyruvic in particular) have been identified.[4, 12] However, the conditions used in these experiments are not necessarily those which exist in the mouth, the plaque, for example, being diluted and out of contact with the surface of teeth and saliva. The time course of the changes in pH of the plaque in the mouth after ingestion of sugars has been thoroughly studied by a number of workers using a variety of technics.[5-10] In brief, the pH always drops rapidly during the first 5 or 10 minutes and then rises slowly to normal.

This report concerns the nature of the acids formed in the plaque in the mouth and the time course of their formation. It is shown that lactic acid accumulates in the plaque after rinsing the mouth with sucrose solution. Furthermore, it is shown that the observed pH drop in plaque can probably be accounted for by the amount of lactic acid which accumulates, at least within a 20-minute interval.

This paper is partially based on a Thesis submitted to the Graduate School of Northwestern University in partial fulfillment of the requirements of the degree of Doctor of Philosophy by Blake W. Moore in September, 1952.

The opinions and assertions of the authors are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

This work was partially supported by grants from the United States Public Health Service.

Received for publication April 14, 1955, revised by author May 15, 1956.

*Present address, Washington University School of Dentistry, St. Louis, Mo.

METHODS

The subjects were recruits from the Great Lakes Naval Training Center. They were 18 to 20 years old, from scattered parts of this country, and on the basis of dental examination by one of the authors (J. K. D.) were considered average or above average in caries rate. Samples of plaque were taken from noncarious areas (chiefly, the labial surfaces of the upper anterior teeth) of each subject before and at 5-minute intervals for 20 minutes after a 1-minute rinse with 50 per cent (w/w) sucrose solution. The pH of each sample was determined with a microglass electrode according to the procedure of Fosdick, Campaigne, and Fancher.[12]

Each sample, having a dry weight between 200 and 800 micrograms, was suspended in 2.0 ml. of 0.04 M cupric sulfate in a 15 ml. centrifuge tube; this was shaken and allowed to extract at room temperature at least 24 hours. The method of Barker and Summerson[13] was used to determine lactic acid. Sample weight was determined by the rapid wet-ash method of Johnson,[14] which is based on the reduction of standard dichromate by the sample. This latter method was used rather than drying and weighing because: (a) it was more rapid; (b) it avoided the possibility of formation or metabolism of lactate during the process of drying and weighing; and (c) it avoided error in weighing caused by contamination of the sample with potassium chloride as a result of the pH measurement. The following was the exact procedure used.

About 0.1 gram of A. R. calcium hydroxide was added to the cupric sulfate extract. After the mixture was shaken and centrifuged, 1.0 ml. of supernatant was transferred to a 150 by 20 mm. test tube. One-tenth milliliter of 0.04 M cupric sulfate and then 5.0 ml. of concentrated sulfuric acid (Baker's Analyzed) were added. The sulfuric acid was dispensed with a calibrated 10 ml. hypodermic syringe fitted with a glass delivery tube. After being mixed, the solution was heated 5 minutes in boiling water and then immediately cooled in ice. Two drops (0.1 ml.) of p-hydroxydiphenyl reagent (as described by Barker and Summerson) were added, the reagent was dispersed by rotating the tube, and the mixture was allowed to stand 30 minutes at 30° C. to develop the color. Then it was heated exactly 90 seconds in boiling water to dissolve excess reagent, and cooled immediately in ice. The optical density at 565 millimicrons was read in a Coleman spectrophotometer. The amount of lactate was found by means of a curve determined with standards prepared from lithium lactate,[15] and also with standards prepared from standard lactic acid (titrated against CO_2-free sodium hydroxide after being boiled to depolymerize).

The residue in the centrifuge tube, after removal of the 1.0 ml. of supernatant for lactate analysis, was used to estimate the sample weight. To the residue 2.0 ml. of 0.0117 M A. R. sodium dichromate (140 microequivalents) in 95 per cent sulfuric acid were added. The mixture was heated in boiling water 30 minutes and then cooled in ice. Ten milliliters of water were added and, after mixing, the precipitate of calcium sulfate was spun down. The calcium sulfate did not adsorb appreciable dichromate. The optical density at 440 millimicrons was read on a portion of the supernatant. The blank was

the dichromate solution diluted as above and then reduced with an excess of solid sodium bisulfite. The number of microequivalents of dichromate reduced by the sample times 7 was taken as the sample weight in micrograms. This is the factor recommended by Johnson and was verified (Table I) by drying samples of plaque on platinum dishes to constant weight in moist air, and then wet-ashing them by the above procedure. The range of sample weights determinable by this method was 200 to 800 micrograms.

TABLE I
COMPARISON OF ACTUAL AND CALCULATED DRY WEIGHTS OF PLAQUE ASSUMING 20 PER CENT HYDRATION AFTER DRYING AND EQUILIBRATING WITH ATMOSPHERIC AIR

ACTUAL WT. (MCG.) (HYDRATED)	0.8 × WT.	OPTICAL DENSITY AT 440 Mμ	CALCULATED WT. (MCG.) FROM OPTICAL DENSITY
800	640	0.258	644
470	376	0.468	371
200	160	0.625	175
650	520	0.340	539
430	344	0.471	371
0	0	0.756	0

It was found, in agreement with Barker and Summerson, that any pyruvate present in the extract was not included in the lactate analysis. For example, 15 micrograms of pyruvate in this procedure gave color at 565 millimicrons equivalent to only 0.4 microgram of lactate. In view of the sensitivity of the lactate analysis, all glassware was cleaned in dichromate sulfuric acid, and then rinsed with tap water, trisodium phosphate solution (to remove adsorbed dichromate), and finally several times with distilled water.

A titration of plaque was done on 500 milligrams (wet weight) of plaque pooled from twenty subjects. Small increments of 1 N lactic acid were added with a fine capillary and the plaque was mixed after each addition. Before the titration was begun and after each addition of lactic acid, a sample of the plaque was taken for pH and lactate determination by the methods described above. The sample was kept at 0 to 1° C. throughout the titration.

RESULTS

The values for pH and per cent lactate (on a dry-weight basis) obtained on 101 subjects are shown in Table II. The data for 8 of the 109 subjects used were discarded because the dry weight of plaque taken from these was outside

TABLE II
AVERAGE pH AND LACTATE AT VARIOUS TIME INTERVALS

	pH				PER CENT LACTATE			
TIME	NO. OF READ-INGS	MEAN	MEDIAN	RANGE	NO. OF READ-INGS	MEAN	MEDIAN	RANGE
Before sucrose rinse	97	6.3	6.3	4.8-7.4	97	0.3	0.2	0.0-1.9
5 minutes after rinse	96	5.7	5.7	4.9-7.3	96	1.5	1.4	0.2-4.1
10 minutes after rinse	99	5.6	5.5	4.6-6.6	99	1.7	1.7	0.0-4.6
15 minutes after rinse	101	5.6	5.7	4.7-6.9	101	1.7	1.6	0.1-5.2
20 minutes after rinse	97	5.8	5.7	4.8-7.0	97	1.5	1.5	0.2-4.1

the 200 to 800 microgram limits. The average pH and average per cent lactate for each time interval are plotted in Fig. 1. The plot shows that, in agreement with other workers, there is a rapid fall in pH in the plaque in the mouth after

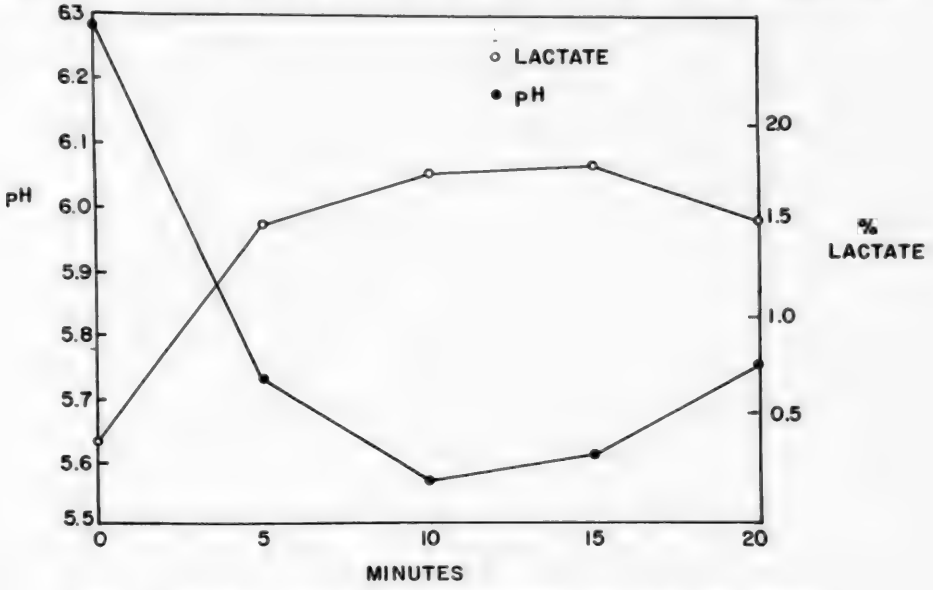


Fig. 1.—Change of plaque pH and per cent lactate after 50 per cent sucrose.

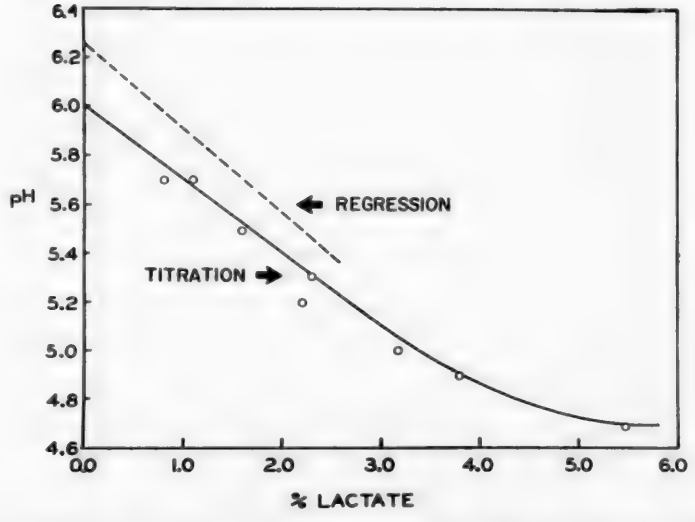


Fig. 2.—Regression of pH on per cent lactate compared with the titration curve of plaque.

the sucrose rinse, which reaches a minimum in 5 to 10 minutes. Furthermore, it is clear that the increase in lactate parallels the pH change.

In order to determine if there was a relationship between the pH and lactate values, the regression of pH on per cent lactate (dry weight) for all 505 points was calculated using the least squares method (Table III). The regression is shown in Fig. 2, along with the experimental titration curve of 500 milligrams (wet weight) of pooled plaque against lactic acid. The straight portion of the titration curve was fitted to the points by the least squares method. Since the slopes of these two curves are similar, all or nearly all of the fall in pH after the sucrose rinse can be accounted for by the observed increase in lactate.

TABLE III
CALCULATION OF CORRELATION AND REGRESSION OF pH ON LACTATE

x = per cent lactate (dry weight)
y = pH

$$\Sigma x = 675.6$$
$$\Sigma y = 2925.9$$
$$Sx^2 = 356.03$$
$$Sy^2 = 144.83$$
$$Sxy = -112.56$$

$$\text{correlation coefficient} = r = \frac{Sxy}{Sx^2 Sy^2} = -0.495 \text{ (sig. at 1\% level).}$$

$$b = \frac{Sxy}{Sx^2} = -0.32$$

$$y = 6.2 - 0.32x.$$

Stralfors[16] has also titrated small samples of plaque with hydrochloric acid. Since in the pH region considered here lactic acid is almost completely ionized, the buffering capacities with hydrochloric and lactic acids can be compared and should be equal. In 8 titrations of plaque, in Stralfors' work, an average of 38 micromoles of HCl per milliliter of plaque were required to reduce the pH from 6.0 to 5.0. If the dry weight of plaque represents about 10 per cent of its wet weight,[17] the amount of lactic acid required to reduce the pH from 6.0 to 5.0, in the in vitro titration reported here, is calculated to be 37 micromoles per milliliter of plaque. Considering the assumptions made, the agreement is very close.

DISCUSSION

The data show that large quantities of lactate accumulate in a short time in plaque in the mouth after sucrose ingestion. However, since only the lactate which accumulated in the plaque was measured, nothing can be inferred about the rate of production of lactate or the rate of removal of lactate by diffusion or metabolism or both, except that the rate of production is at least as great as the measured rate of accumulation.

On the basis of these experiments, it is probable that the observed decrease in pH can be accounted for quantitatively by the amount of lactate which accumulates, at least during the 20-minute period studied, which in most instances includes the time of minimum pH. This suggests that either negligible amounts of any other acids accumulate during this period, or if they do build

up in quantity, they are weak acids compared to lactic ($pK_1 = 3.86$). It is, therefore, improbable that appreciable amounts of pyruvate ($pK_1 = 2.57$), citrate ($pK_1 = 3.1$), and acetate ($pK_1 = 4.73$) accumulate in plaque. This conclusion that lactic acid is probably the only important cause of the pH drop in plaque in the mouth differs from some of the in vitro results reported by other workers on saliva[18-21] and on plaque.[4, 12] Many factors in this complex process which are possibly involved in determining the rate and type of metabolism of sucrose and lactate in the mouth are not necessarily duplicated in vitro. These factors may include contact of the plaque with the enamel and with saliva, continuous diffusion of sucrose into the plaque and of acids out of the plaque, the oxygen tension in the plaque, as well as the species of microorganisms inducing the change. In the experiments described here, the natural conditions are not altered. On the other hand, this method suffers from the disadvantage of having to take the several samples of plaque from different parts of the mouth, because of its scarcity. The great spread of the data, even within a series of samples from the same subject (Table II), is probably related to this. It is probable that plaques in different parts of the mouth differ in their contact with saliva, in their water content, in buffering capacities, and in their microbial populations. However, this defect is minimized by statistical treatment of the data when large numbers of individuals are used.

The finding that appreciable amounts of a rather strong acid, lactic, accumulate in plaque in vivo, together with the earlier results on pH changes in the plaque in vivo, support the theory that decalcification by acids is primary. The demonstration that lactic acid is the major acid formed in vivo supports the theory that the acids arise by fermentation of carbohydrates and not by some other process. Therefore, this work provides further evidence, although indirect, in favor of Miller's theory.[22]

Table IV shows the distribution of minimum pH values reached for each individual during the 20-minute interval. Enamel decalcifies at comparable pH values in buffers and in saliva in vitro.[23-26] Furthermore, lactate ion forms soluble complexes with calcium ion[27-29] and, therefore, may also have an effect on enamel similar to that to hydrogen ion. If the 2 equilibria

$$(1)\quad H^+ + L^- \rightleftharpoons HL$$
$$(2)\quad Ca^{++} + L^- \rightleftharpoons CaL^+$$

are considered, it can be shown that

$$\frac{(CaL^+)}{(Ca^{++})} = \frac{K_1K_2c}{(H^+) + K_1},$$

where K_1 is the ionization constant for lactic acid with the value of 1.4×10^{-4}, K_2 is the dissociation constant for the complex with the value of 6.6,[29] and c is the total concentration of lactate. At pH 5.0 and lactate concentration (wet weight) of 0.35 per cent, $\frac{(CaL^+)}{(Ca^{++})}$ has the value 0.16, showing that appreciable amounts of calcium ion can be complexed by the lactate formed under in vivo conditions in plaque.

TABLE IV
DISTRIBUTION OF MINIMUM pH VALUES

MINIMUM pH	NO. OF SUBJECTS
below 5.0	16
5.0 to 5.2	26
5.3 to 5.5	29
5.6 to 5.8	19
above 5.8	11
Total	101

However, since the demonstrated changes in pH and lactate contents in plaque are transient changes, the important factor in caries would be the rate of decalcification of enamel and not necessarily values at equilibrium. Very little is known about the rate of decalcification of enamel under natural conditions. Isotope studies using P^{32} show that exchange of phosphate occurs in vitro between enamel and buffers or saliva, and in vivo between enamel and saliva;[30-33] these results support the possibility of decalcification under certain conditions. Direct confirmation of Miller's theory must be derived from measurements of the rate of decalcification of enamel under various conditions, or from experiments which demonstrate directly whether enamel does decalcify in the mouth in response to increase in acid concentration.

Differences in susceptibility to caries may arise from differences in resistance of the teeth and from differences in acid accumulation or buffering capacity of plaques. Since the method described here measures both acid accumulation and buffering capacity, it provides a tool for determining the influence of plaque itself on susceptibility to caries.

SUMMARY

1. A method for simultaneous measurement of pH and lactate content of dental plaque in situ is described.

2. During a 20-minute interval following a 50 per cent sucrose mouth rinse, the increase in per cent lactate paralleled the decrease in pH.

3. All or nearly all of the observed decrease in pH can be accounted for quantitatively by the increase in lactate.

REFERENCES

1. Henderson, H., and Millet, J. A. D.: On the Hydrogen Ion Determination of Normal Saliva, *J. Biol. Chem.* **75**: 559, 1927.
2. Dewar, M. R.: Some Observations on the pH of Saliva, *D. J. Australia* **21**: 113, 1949.
3. Haggard, H. W., and Greenberg, L. A.: The Acidity of Saliva After Ingestion of Carbohydrate and Acid-Containing Substances, *J. D. Res.* **30**: 126, 1951.
4. Muntz, J. A.: Production of Acids From Glucose by Dental Plaque Material, *J. Biol. Chem.* **148**: 225, 1943.
5. Stephan, R. M.: Hydrogen-ion Concentration of the Dental Plaque, *J. D. Res.* **17**: 251, 1938.
6. *Idem:* Changes in Hydrogen-ion Concentration on Tooth Surfaces and in Carious Lesions, *J. A. D. A.* **27**: 718, 1940.
7. Stephan, R. M., and Miller, B. F.: Quantitative Method for Evaluating Effectiveness of Physical and Chemical Agents Which Modify Hydrogen-ion Concentration on Tooth Surfaces. Observations on Effects of Synthetic Detergents and Urea, *J. D. Res.* **21**: 315, 1942.
8. *Idem:* The Effect of Synthetic Detergents of pH Changes in Dental Plaques, *J. D. Res.* **22**: 53, 1943.

9. Stephan, R. M.: Intra-Oral Hydrogen-ion Concentrations Associated With Dental Caries Activity, *J. D. Res.* **23**: 257, 1944.
10. Stralfors, A.: Studies of the Microbiology of Caries II. The Acid Fermentation in the Dental Plaques in Situ Compared with *Lactobacillus* Counts, *J. D. Res.* **27**: 576, 1948.
11. Miller, B. F., Muntz, J. A., and Bradel, S.: Decomposition of Carbohydrate Substrates by Dental Plaque Material, *J. D. Res.* **19**: 473, 1940.
12. Fosdick, L. S., Campaigne, E. E., and Fancher, O.: Rate of Acid Formation in Carious Areas: The Etiology of Dental Caries, *Ill. D. J.* **10**: 85, 1941.
13. Barker, S. B., and Summerson, W. H.: The Colorimetric Determination of Lactic Acid in Biological Material, *J. Biol. Chem.* **138**: 535, 1941.
14. Johnson, M. J.: A Rapid Micromethod for Estimation of Non-Volatile Organic Matter, *J. Biol. Chem.* **181**: 707, 1949.
15. Hillig, F.: The Colorimetric Determination of Lactic Acid in Milk and Milk Products, *J. Assoc. Offic. Agric. Chem.* **20**: 130, 1937.
16. Stralfors, A.: Studies of the Microbiology of Caries III. The Buffer Capacity of Dental Plaques, *J. D. Res.* **27**: 587, 1948.
17. Blackwell, R. Q.: Northwestern University. Personal Communication, 1954.
18. Fosdick, L. S., and Wessinger, G. D.: Carbohydrate Degradation by Mouth Organisms. II. Yeast, *J. A. D. A.* **27**: 203, 1940.
19. Summerson, W. H., and Neuwirth, I.: The Production of Acids From Glucose by Oral Microorganisms, *J. D. Res.* **20**: 157, 1941.
20. Neuwirth, I., and Summerson, W. H.: The Utilization of Lactic and Pyruvic Acids by Oral Microorganisms, *J. D. Res.* **21**: 241, 1942.
21. *Idem:* The Production of Acids From Glucose by Oral Microorganisms: Lactic and Pyruvic Acids, *J. D. Res.* **30**: 100, 1951.
22. Miller, W. D.: The Microorganisms of the Human Mouth, Philadelphia, 1890, S. S. White.
23. Enright, J. J., Friesell, H. E., and Trescher, M. O.: Studies of the Cause and Nature of Dental Caries, *J. D. Res.* **12**: 759, 1932.
24. Karshan, M., and Rosebury, T.: Relationship of pH, Ca, and P to Solubility of Enamel, *J. D. Res.* **14**: 220, 1934.
25. Fosdick, L. S., and Starke, A. C., Jr.: Solubility of Tooth Enamel in Saliva at Various pH Levels, *J. D. Res.* **18**: 417, 1939.
26. Ericsson, Y.: Enamel-Apatite Solubility, *Acta odont. scandinav.* **8**: Suppl. 3, 1949.
27. Cannan, R. K., and Kibrick, A.: Complex Formation Between Carboxylic Acids and Divalent Metal Cations, *J. A. Chem. Soc.* **60**: 2314, 1938.
28. Joseph, N. R.: The Dissociation Constants of Organic Calcium Complexes, *J. Biol. Chem.* **164**: 529, 1949.
29. Schubert, J., and Lindenbaum, A.: Stability of Alkaline Earth-Organic Acid Complexes Measured by Ion Exchange, *J. A. Chem. Soc.* **74**: 3529, 1952.
30. Volker, J. F., and Sognnaes, R. F.: A Study of the Phosphorus Metabolism in the Dental Tissues of a Cat by the Use of Radioactive Phosphorus, *J. D. Res.* **19**: 292, 1940.
31. *Idem:* The Distribution of Radioactive Phosphorus in the Teeth of Experimental Animals, *J. D. Res.* **20**: 471, 1941.
32. Pedersen, P. O., and Schmidt-Nielsen, B.: Untersuchungen über Stoffwechsel in den Zahnhartgeweben menschlichen Zähne unter Anwendung radioaktiven Phosphors als Indikator, *Schweiz. Monatsschr. Zahnhlk.* **51**: 647, 1941.
33. Berggren, H.: Mineral Metabolism in the Dental Hard Tissues, *Acta Radiol.* **27**: 248, 1946.

THE FORMATION OF LACTIC ACID IN DENTAL PLAQUES

II. Oral Conditions of Primitive Bushmen of the Western Kalahari Desert

A. J. CLEMENT, R. PLOTKIN, AND L. S. FOSDICK

University of the Witwatersrand, South African Institute for Medical Research, Johannesburg, Union of South Africa, and Northwestern University, Chicago, Ill.

ALTHOUGH there have been numerous studies of the incidence of dental caries on some of the rather primitive African natives,[1] there have been few studies concerning the nomadic, primitive Bushmen. Clement[2] described the oral conditions of about 40 Bushmen of the Kukong Pan and found that 90 per cent of the individuals were caries-free, and that of the 10 per cent that did have carious lesions there was an average of 1.5 carious teeth per mouth. Furthermore, 2 carious lesions were the most found in any one month.

In view of this situation, another expedition was organized in order to obtain more complete clinical, chemical, and bacteriologic data on the dental aspects of primitive Bushmen.

With the possible exception of the Australian Aborigines, the Bushmen are probably the most primitive of mankind. They are a nomadic group, perhaps of so-called Mongolian descent, and are possibly the original inhabitants of South Africa. During the past few centuries they have been driven into the more inaccessible areas of the Kalahari Desert, first by the white man from the south and then by the black man from the north. Under these circumstances, the Bushmen either had to retreat to the areas where neither the white nor the black man would live or they had to become assimilated. Even today the black natives of Bechuanaland have Bushmen slaves that eventually become absorbed by the Bantu tribes of the country.

In so far as the Bushman is nomadic, primitive, and very timid, except for those who have adopted the native ways and customs or are slaves of the black natives one ordinarily never sees one except by chance. Due to some unusual circumstances* and to the excellent cooperation of the Bechuanaland Administration, it was possible for the authors to obtain 50 primitive Bushmen in the Ghanzi area for this study.

The Bushman is rather short, slight of stature, and of exceptional stamina. The skin is dry, with practically no body or facial hair. The scalp hair is tightly coiled into spiral knots (pepper corn). The eyes have a moderate slant, and the nose is low and rather broad at the base. The ears show little or no signs of lobes. Although the Bushman is usually rather slender, there is a

Received for publication April 14, 1955, revised by authors May 15, 1956.

*Two Englishmen, the Ramsden brothers, settled near the Bushman territory in 1914, and during the ensuing period have gained the confidence of the Bushmen. Arrangements were made through them for the Bushmen to meet at 2 locations so that we could study them. They also furnished interpreters.

marked tendency for fat to accumulate on the thighs and buttocks (steaptogyia), particularly in women. In the adult members there is either a protuberant belly or loose, folded skin, resulting from alternate gorging and starvation. He has a very characteristic and unpleasant odor.

The Bushman's diet consists of whatever he can find to eat, and he practices no form of agriculture, nor does he provide in any way for the future. As animal life is very profuse, he can usually find game; however, he may have considerable difficulty in making the kill. He uses a very crude bow and arrow for hunting. The arrow is usually tipped with poison which he prepares from beetles, vegetable juices, or poisonous snakes. Because of the primitive bow, he must shoot at very close range in order to be effective. (Any animal with a normal olfactory sense can probably smell a Bushman before he is close enough for the arrow to penetrate.) He also makes snares and, in the Okavanga region, is said even to catch fish.

In addition to the large game, the Bushman eats ants, grubs, and other protein animal life. In so far as the desert abounds in melons and berries, these are a major part of the diet in season. He also eats the roots of certain plants. The Bushman knows how to cook food; often he cooks the melons before they are eaten. In so far as he has no cooking utensils, his food is contaminated with the fine, hard desert sand, which probably explains the marked degree of tooth abrasion characteristic of all primitive Bushmen.

From a nutritional point of view this diet might be quite satisfactory, if the above constituents were always present; however, this is not the case. When berries and melons are in season these are the major items of diet. When these foods are not in season, then the diet is roots and meat. A fair portion of the time nothing is available, so the Bushmen gorge themselves when food is available and half starve when it is not. Thus, the diet of the Bushmen may be considered suboptimal by any recognized standard.

The present study was conducted on 50 primitive Bushmen, 21 males and 29 females. The ages varied from about 8 years to rather old individuals. There was no way of telling the exact age. The approximate age of the children could be rather accurately determined on the basis of the eruption schedule, which is slightly earlier than in civilized man. (The only sequential difference is that the premolars erupt after the second molars.) The age recorded for the adults was merely a rough estimation.

The teeth were examined for dental caries by mouth mirror and explorers. The condition of the soft tissues was recorded as –, +1, +2, +3, and +4, according to the degree of severity of the gingivitis. The observation was entirely subjective. Calculus was also recorded in the same manner, as was the degree of abrasion. Any missing, broken, or abscessed teeth were also noted.

When the clinical examination was completed the pH of the dental plaque on the proximal and buccal surfaces of the upper premolars was measured. The pH was taken by means of an antimony electrode prepared according to the method of Forscher.[3] After the pH was measured, a sample of the plaque that had been under the antimony electrode was removed and dropped into

2 c.c. of 1 per cent copper sulfate solution. The mouth was then rinsed for 30 seconds with a 50 per cent sucrose solution, after which the pH measurements and plaque samples were taken at 5-minute intervals for 20 minutes. The pH curves were not obtained on the first 4 cases, but samples of plaque were obtained from these individuals. The pH measurements and plaque removal were omitted at the 20-minute interval in 15 cases.

After the pH curves were secured each individual was given a stick of paraffin to chew in order to stimulate saliva. A sample of saliva was collected for the Snyder test, lactobacillus counts, buffering capacity, and methylamine determinations and for bacteriologic study. The Snyder test, the lactobacillus counts, and the buffering capacity of the saliva were performed on the spot, a small tent serving for a bacteriology laboratory. The samples of dental plaque were preserved in copper sulfate and analyses were performed later. The saliva for the methylamine test was preserved in alcoholic hydrochloric acid. Smears for further bacteriologic investigation were taken. This paper deals with a short summary of the clinical findings, along with the tests for caries activity and the pH and buffering capacity of the dental plaques.

RESULTS

Not a single definite carious lesion was found in the teeth of the 50 people examined. In 7 of the cases hard, glassy, black spots were found on the occlusal surfaces of 9 of the molar teeth. In all of these cases there was considerable abrasion. These spots were where the pits or grooves of the occlusal surfaces of the molars would be if the abrasion had not worn the cusps down to where grooves were practically eliminated. If these spots had at one time been carious, the lesions were certainly arrested, probably because of the abrasion, and at no time could they have been very active (Table I).

TABLE 1
AVERAGE pH AND LACTATE AT VARIOUS TIME INTERVALS*

	pH				PER CENT LACTATE			
TIME	NO. OF READINGS	MEAN	MEDIAN	RANGE	NO. OF READINGS	MEAN	MEDIAN	RANGE
Before sucrose rinse	46	6.9	6.9	6.1-7.8	50	0.2	0.2	0.1-1.2
5 minutes after rinse	46	6.6	6.6	5.8-7.6	48	0.5	0.4	0.1-1.9
10 minutes after rinse	46	6.5	6.4	5.7-7.8	49	0.5	0.4	0.1-1.8
15 minutes after rinse	46	6.5	6.4	5.7-7.5	47	0.6	0.5	0.1-1.8
20 minutes after rinse	31	6.6	6.7	5.6-7.6	36	0.5	0.5	0.2-1.1

*The individual data on the 50 Bushmen examined are available from the authors.

The abrasion was marked in all adult cases and even the teeth of the children were slightly abraded. In the older adults the abrasion was so marked that the dentin was exposed. The exposed dentin was hard and glossy. In no case was erosion observed.

The oral hygiene in general was not as poor as would be expected. It was observed that occasionally a Bushman would place a finger in the ashes around the campfire and rub them over his teeth and gums. This is believed to be the only form of oral hygiene practiced. Calculus was most profuse. In the very

young it was ordinarily not marked, but in most of the adults large crusts were observed. The excessive calculus is probably linked with high alkali mineral content of the Pan water they drink.

The gingiva was, in general, in very poor health. In most cases gingivitis and even periodontitis was the rule. In only 4 cases was the gingiva healthy. Three of these 4 cases were children; the one lone case of normal gingiva in the adult was that of a young female. In 8 cases the gingivitis was mild; all but 2 of these were also in young adult females. The cause of the marked gingivitis is not known, but excessive calculus around the teeth and the sand and alkali of the desert that contaminate most of the cooked food may be partially responsible.

It was quite surprising that most of the adults examined had full complements of teeth, in view of the periodontal condition that persisted. Teeth were missing in 7 of the individuals, in 4 of whom the teeth were broken. In the other cases they were probably lost because of periodontal conditions. In one case 3 teeth were missing, and in another case 3 teeth were broken. In one case an abscess was present. A couple of those present were not used for pH measurements because of insufficient teeth; thus, missing teeth in general are more prevalent than the data presented would indicate. Furthermore, as many of them had to travel long distances by foot to reach our locations, many of the less healthy were not secured.

It is interesting to note that in no case did the indicator change in the Snyder test previous to 48 hours. Seven of the 50 changed after 3 days' incubation. The lactobacillus counts were not satisfactory. Most of the samples were overgrown with mold. This was probably due to the winds that predominate in the desert along with inadequate laboratory shelter. Those that were not overgrown with mold had no lactobacillus colonies present.

In view of the above, it is quite obvious that this group of individuals is not caries-active. The clinical examinations and the laboratory tests for caries activity check remarkably well. This is quite unusual, in view of the results obtained by the acid neutralizing activity of the saliva, which was measured in 27 individuals. It was found that the range of acid neutralization was about the same as that for dental students, but that the average was slightly less.

The pH of the dental plaque before and after a sugar rinse was quite unique. In only one case did the pH drop as low as 5.7, and in 82 per cent of the cases the pH did not drop below 6.0.

The lactate content of the dental plaque after a sugar rinse is still more unusual. The average lactate content of the dental plaque at none of the time intervals exceeded 0.58 per cent. This compares with 1.7 per cent in the caries-active white people. The maximum lactate found in any plaque was 1.9 per cent. Lactate in excess of 1 per cent was found in only 13 plaque samples of the 234 analyzed.

DISCUSSION

It has long been thought by many investigators that the carious lesion is initiated by acid formed by fermentable carbohydrate on the tooth surface. It

has been repeatedly shown that when fermentable sugars are taken into the mouth of caries-active individuals, acid rapidly forms in the carious lesions and in the dental plaques. It has also been quite conclusively shown that in caries-inactive white people when sugars are placed on the tooth surface acids also form but in smaller amounts than in caries-active plaques. Furthermore, the acid potential formed on the tooth surface in caries-active cases is greater than that in caries-immune plaques. The high pH values and the low total lactic acid in the Bushman dental plaques certainly follow the pattern for caries-inactive individuals.

One might inquire, why do the Bushmen not have dental caries? Their diet is not of the best. Although they do have a rather high protein diet at times, during certain seasons of the year the diet is rather high in fermentable sugar. The fruits and melons are ordinarily about 10 per cent sugar and roots are predominately starch and cellulose. It is true that all of the sugars consumed are natural, but still fermentable. Furthermore, the fruits and berries are predominately acid.

It is quite probable that concentration of the sugar is the all-important factor in regard to the initiation of dental caries. If this is the case, then one would not expect fruit juices, acid sweet fruits, or their juices (or even acid sweet carbonated beverages), to initiate a carious lesion; but once a lesion is established, all sugars and acid foods, regardless of concentration, would cause a continuation of this process.

Another phenomenon that may be of considerable significance in regard to the caries inactivity of Bushmen is that described by Jay.[4] He found that when individuals abstain from sugars for relatively short periods of time, the lactobacillus soon disappear from the mouth. After this has been accomplished the individual may return to a diet containing considerable fermentable sugar without suffering a rapid return to acidogenic oral flora. In the case of the Bushmen there is an annual period of abstinence from fermentable sugars and the diet, aside from starch of roots, is almost exclusively protein in nature. Thus, with the periodic forced abstinence from fermentable sugars, the Bushmen may never attain a cariogenic oral flora.

The data that were secured indicate that the acid-neutralizing power and flow of saliva are not unlike those in normal caries-active saliva. The buffering capacity of the dental plaques is in the same order of magnitude as, or slightly less than that found in caries-active white people. The diet sporadically contains appreciable amounts of fermentable sugars, but the oral flora is not predominately acidogenic. Thus, it would seem that for some reason or other the oral flora is not similar to that of caries-active individuals, but is rather like that of caries-inactive individuals in respect to acidogenic microorganisms.

It may well be that the Bushman has little natural protection against dental caries, but in so far as he never receives any concentrated sugar he has no need for protection. It is quite possible also that if he were furnished the attack potential in the form of concentrated sugar, many of these people would have rampant caries within a short time.

It would be interesting to secure two groups of Bushmen and provide one group with dilute sugar solutions for at least a year while a comparable group is provided with concentrated sugar. This would either prove or disprove the idea that the refining of sugar provides a source of concentrated sugar and that only concentrated sugar can initiate a carious lesion.

SUMMARY

Clinical examinations were made of 50 primitive Bushmen for the incidence of carious lesions, calculus, and periodontal disturbances. It was found that these individuals are caries-inactive, the oral hygiene is poor, calculus is marked, and periodontal disease is rampant.

Laboratory tests indicate that the oral flora is not acidogenic and that after a sugar rinse the dental plaques do not become sufficiently acid to cause decalcification. The buffering capacity of the dental plaques and saliva is not markedly different from that found in white caries-active cases.

We wish to express our thanks to the Bechuanaland Administration and to the South African Institute of Medical Research for their active participation and cooperation in this project and to the Council of Scientific and Industrial Research, South Africa, for their financial assistance.

REFERENCES

1. Clement, A. J.: The Basic Aetiology of Dental Caries With Special Reference to Unique Opportunities for Caries Research in South Africa, *Off. J. D. A. South Africa* **7**: 180, 1952.
2. *Idem:* Clinical Findings on Bakalagadi Children and Various Bushmen of South Africa, *Off. J. D. A. South Africa* **8**: 50, 1953.
3. Forscher, B. K., Paulsen, A. G., and Hess, W. C.: An Improved Micro Antimony Electrode and pH Determination in the Periodontal Pocket, *J. D. Res.* **32**: 646, 1953.
4. Jay, P.: Reduction of Oral Lactobacillus Acidophilus Counts by Periodic Restriction of Carbohydrate, *Am. J. Orthodontics* **33**: 162, 1947.

THE FORMATION OF LACTIC ACID IN DENTAL PLAQUES
III. Caries-Immune Individuals

HAROLD R. ENGLANDER, WM. J. CARTER,* AND LEONARD S. FOSDICK

The Dental Research Facility, U. S. Naval Training Center, Great Lakes, Ill., and Northwestern University Dental School, Chicago, Ill.

THE dental plaques of caries-active individuals and caries-free primitive men have been investigated.[1, 2] In both studies the pH and lactate content of the plaques were measured.

This paper deals with the pH and lactate content of the plaques from caries-free Americans before and during the 20-minute period that followed a 1-minute 50 per cent sucrose rinse. The analytic procedures used in these tests are exactly the same as previously used on the plaques from caries-active individuals. With the data available on caries-active cases, primitive man, and caries-immune individuals, a discussion of the differences among the data is presented.

EXPERIMENTAL PROCEDURE

Fifty-one subjects were selected from the personnel at the Great Lakes Naval Training Center. They ranged in age from 17 to 23 years and, in general, were from the central and southwestern sections of the United States. On the basis of clinical and radiographic dental examination, they were considered completely caries-free. In order to be classified as completely caries-immune, it was required that each subject be free of caries and previous dental restorations. Furthermore, he must be caries-inactive as determined by salivary lactobacillus counts according to the method of Jay.[3] Considerable difficulty was experienced in securing individuals of this type, as they are extremely rare. Approximately 50,000 recruits were screened before the 51 cases were obtained. (About 50 per cent of these caries-immune individuals showed clinical evidence of mild mottled enamel.)

Samples of dental plaque were removed from the teeth of each subject before and at 5, 10, 15, and 20 minutes after 1-minute 50 per cent sucrose rinse. The samples of plaque were removed from the gingival areas of the buccal surfaces of the upper or lower molars. Whenever possible, all samples of plaque were taken from the same tooth. (From a physical and chemical point of view, and for purposes of this paper, plaque is designated as the organic coating of the tooth, regardless of its physical properties or derivation. This

Presented at the March, 1954, meeting of The International Association for Dental Research, French Lick, Ind. (*J. D. Res.* **33**: 656, 1954.)

The opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Department of the Navy, or the Naval Service at large.

Received for publication April 14, 1955, revised by authors May 15, 1956.

*Present address: The University of Kansas City School of Dentistry, Kansas City, Mo.

would include film, materia alba, and any matter that is consistently found on the tooth surface.) Each sample was placed between a glass electrode and a calomel half cell, as described by Fosdick, Campaigne, and Fancher.[4] The pH of each sample was measured by means of a Coleman vacuum tube potentiometer.

After the pH was determined, the sample of plaque was teased from the capillary of the calomel half cell and placed into 2 c.c. of 1 per cent copper sulfate solution, to stop all enzyme action and preserve it. This sample was then used for the determination of total lactate by a modified method of Barker and Summerson[5] and for a determination of the weight. The procedures used are described by Moore, Carter, Dunn, and Fosdick.[1]

RESULTS

The complete results are shown in Table I. As can be seen from this table, the results are quite consistent. In no case did a high acidity develop. The minimum pH attained by any of the plaques was pH 5.5, and this only occurred in 2 of the 51 cases. The average minimum pH was 6.4.

TABLE I
AVERAGE pH AND LACTATE AT VARIOUS TIME INTERVALS

	pH				PER CENT LACTATE			
TIME	NO. OF READINGS	MEAN	MEDIAN	RANGE	NO. OF READINGS	MEAN	MEDIAN	RANGE
Before sucrose rinse	50	6.8	6.9	5.6-7.8	50	0.4	0.3	0.0-2.2
5 Minutes after rinse	51	6.7	6.7	5.5-8.0	51	0.6	0.5	0.1-1.7
10 Minutes after rinse	48	6.7	6.7	5.5-8.0	48	0.7	0.5	0.0-1.9
15 Minutes after rinse	49	6.7	6.8	5.6-7.9	49	0.7	0.5	0.1-2.0
20 Minutes after rinse	45	6.8	6.9	5.7-7.8	45	0.6	0.6	0.0-1.5

Although in no case did the pH drop below 5.5, the lactate concentration in the immune plaque was considerable. The average maximum lactate formed during the 20-minute interval after the application of sugar was 0.87 per cent on a dry weight basis. This is far in excess of that required to cause decalcification if no neutralization has taken place.

DISCUSSION

The rate at which acid forms on the tooth surface is governed primarily by the adequacy of the substrate and the enzyme system. Thus, if both an ideal substrate and an ideal enzyme system, including all of the enzymes, coenzymes, and mineral activators, are present, pyruvic and lactic acid can form with extreme speed. Sucrose is an excellent substrate for many microorganisms. The enzyme system on the tooth surface is probably never ideal. The enzymes can and do come from the microorganisms resident in the plaque. Many of the coenzymes can be synthesized by some of the oral organisms, but many of the acidogenic organisms are dependent upon the diet of the individual for both mineral and coenzyme factors. Many of the acidogenic microorganisms do not have ideal enzyme systems when in pure strain and many dental plaques do not have a microbial flora furnishing efficient enzyme systems for glycolysis.

It is known that harmful acidities are almost invariably formed in carious lesions in the presence of sucrose. In caries-active mouths harmful acidities are frequently present in some of the dental plaques after a sugar rinse. In the average young American adult it is possible to find a spectrum of pH values in different plaques from different teeth and different areas of the same tooth. However, when the plaques on the same areas are measured at different times, remarkably consistent results are obtained.

There has been considerable theoretical and laboratory work done in attempts to determine the "critical" pH, or that value above which the teeth are safe and below which the teeth are harmed. From theoretical considerations a pH of 5.2 may be considered harmful in normal saliva, but actual experiments indicate that values above this may also be harmful. Because of the variations that normally occur in the mouth no absolute value above pH 5.2 can be designated. Perhaps by comparing the pH values attained in caries-active and caries-immune mouths a better conception of the safe acidities can be obtained.

If the acid formed from fermentable carbohydrates is the immediate cause of dental caries, then one would expect to find the acid only in those areas that are involved in the carious process. It is logical to suppose that if all the surfaces of all the teeth of an adult 17 to 23 years of age had withstood their oral environment during the lives of the teeth, and that under the circumstances a nonacidogenic flora had been predominant, no harmful acidities would be likely to occur after the sugar rinse. Thus, one would expect that in general even the maximum acidities found in the immune mouths would be considered harmless. Similar reasoning could be applied to the Bushmen.[2]

On the other hand, in the case of caries-active subjects, one might say that those areas over which a plaque exists would be more likely to decay. Harmful acidities would be expected only in those plaques under which a cavity would subsequently occur. It is not known how long it takes to initiate a lesion so the number of areas in which harmful acidities might develop is uncertain but it is logical to suppose that the number would be at least as great as the annual increment of new lesions and probably greater.

TABLE II
AVERAGE pH AND LACTATE AT TIME INTERVALS

	BEFORE SUCROSE			MINUTES AFTER SUCROSE RINSE											
				5			10			15			20		
		LACTATE			LACTATE			LACTATE			LACTATE			LACTATE	
	pH	%	mM/l	pH	%	mM/l	pH	%	mM/l	pH	%	mM/l	pH	%	mM/l
Bushmen	6.9	0.2	26.4	6.6	0.5	51.9	6.5	0.5	55.3	6.5	0.6	63.6	6.6	0.5	52.3
Caries-immune	6.8	0.4	42.5	6.7	0.6	71.2	6.7	0.7	77.5	6.7	0.7	72.8	6.8	0.6	66.2
Caries-active	6.3	0.3	43.0	5.7	1.5	164.2	5.6	1.7	188.0	5.6	1.7	191.8	5.8	1.5	166.4

The average pH at the various time intervals on the Bushmen, caries-immune, and caries-active cases (see Table II) indicates that there is a wide variation between caries-active and caries-immune individuals. The greatest average acidity is at the 10 and 15 minute intervals. However, the average

values at the various time intervals do not accurately reflect true conditions. Due to the relation between acidity and the rate of decalcification the average pH is not very revealing. The maximum acidity attained in each case would be a more accurate reflection of conditions (Table III). Even here averages may confuse the issue, if the decalcification process is dependent upon a critical value.

TABLE III
AVERAGE MINIMUM pH AND MAXIMUM LACTATE

	AVERAGE MINIMUM pH	AVERAGE MAXIMUM mM/1 LACTATE
Bushmen	6.3	95.5
Caries-immune	6.4	107.4
Caries-active	5.4	244.7

If we assume that the caries-immune cases should never have harmful plaque acidities while the plaques from caries-active people should have only selected areas of harmful acidities, then the distribution of the maximum acidities attained during the 20-minute interval should be significant. Fig. 1 shows the distribution curves of the maximum acidities attained in the 3 types of patients. It is interesting to note that the value most frequently attained by the immune cases and Bushmen is pH 6.4 to 6.5, while that of caries-active cases is 5.2 to 5.3. The lowest values obtained in the caries-immune cases and Bushmen were 5.4 to 5.5, and these occurred in only 2.5 per cent of the cases. Values below this range were obtained in the caries-active subjects in 57 per cent of the cases. If the above reasoning is correct, one might infer that acidities of pH 5.4 or above are harmless while values below this may be harmful. This might indicate that only in 57 per cent of the caries-active cases were precarious plaques studied.

THE LACTIC ACID IN THE DENTAL PLAQUES

Although the rate of lactic acid formation is governed by 2 primary variables, the amount of lactic acid and salts present in the plaques is regulated by many factors. The amount of lactate present at any time is the result of the rate of lactic acid formation, the rate at which it or its salts diffuse from the plaque, and the rate at which it, or its salts, are metabolized by the microorganisms in the plaque. If lactic acid is the only acid formed, as suggested on the basis of titration curves,[1] then the pH of the plaque would be a function of the lactate present, provided the diffusion of the acid and salts and the neutralization and metabolism of the acid were the same in each type of plaque. In view of the many variables present the average lactate as determined by analysis was recorded at the same pH levels as those indicated in Fig. 1. These were placed on a bar graph (Fig. 2). This yields some very surprising results. The amount of lactate present at equivalent pH values does not correlate well with caries activity. At pH 6.6 to 6.7 the caries-active cases have 200 mM per kilogram of lactate, while at the same pH level the caries-immune plaques and those of the Bushmen have only 60 mM per kilogram. At

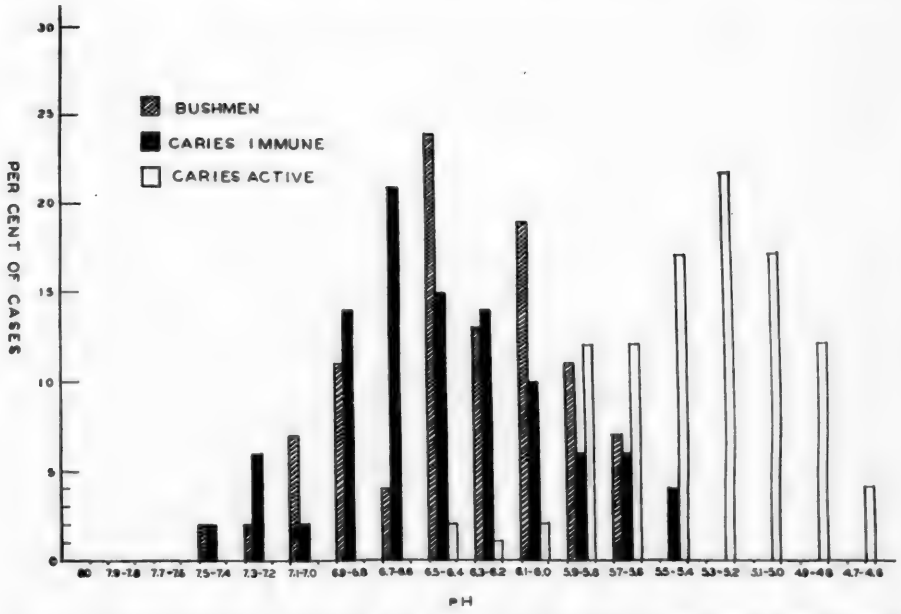


Fig. 1.—The distribution of minimum pH values attained during a 20-minute interval after a sugar rinse.

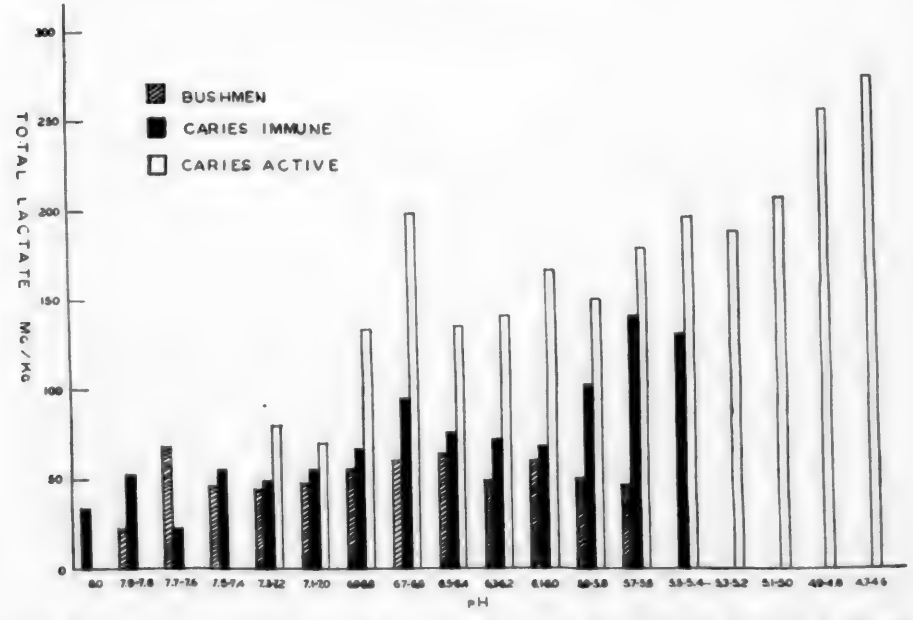


Fig. 2.—The average lactate content of dental plaques as determined by analysis at the pH levels attained after a sugar rinse.

the other pH levels in both caries-active and caries-immune plaques the ratios are similar. This would indicate that the caries-immune cases cannot tolerate as much lactate as the caries-active cases without a lowering of the pH value. Thus, it would seem that our caries-immune plaques do not have as great a neutralization action as the caries-active plaques, or the diffusion is such that it appears so. There is some indication that the latter is probably the case. There does not seem to be a correlation between the caries-immune plaques and caries-active plaques when the lactates at each pH level are averaged.

THE STATE OF LACTIC ACID IN THE DENTAL PLAQUE

Unless there are some extenuating circumstances such as an insoluble surface formed, the effect of acids on enamel is associated with the pH of the acid solutions. The H^+ of weak acids can be calculated if the concentration of the acid and the ionization constant are known; $H^+ = \sqrt{Ka \times \frac{g\ acid}{M}}$. Calculations based on the average maximum lactate (Table III) and the assumption that the plaque is 90 per cent water, indicate that the pH of the plaques would be 2.74, 2.91, and 2.94 for the caries-active, caries-immune, and Bushmen, respectively, if none of the formed acid was neutralized. This is indeed far in excess of the acidity necessary for decalcification. It is obvious from the pH values attained by actual measurement that in all cases large quantities of the acids that formed are immediately neutralized in the plaques. The neutralization could occur from the saliva or from the buffering substances in the plaque itself. The amino acids and amino groups which are present in the plaque could account for some of the neutralization. It would be of considerable interest to determine the exact source of the neutralization effects, but from the data available this is impossible. Even though the source of the neutralization cannot be ascertained it is possible to determine how much of the acid has been neutralized.

Regardless of the other acids that may be present, and regardless of the amount of lactate diffused from the plaque, the state of the lactate that remains in the dental plaque is a function of the H^+ and may be expressed by the Henderson-Hasselbalch equation $pH = pK_1 + \log\frac{salt}{acid}$. The total lactate may be considered salt + acid or neutralized acid + free acid. In so far as the pH and lactate were analytically determined on the same sample except in the case of the Bushmen, the equation should be quite accurate. When the lactate values as determined analytically are subjected to the equation, the results are quite revealing. A smooth curve is obtained (Fig. 3). This curve, obtained by using the analytical values of total lactate and the measured pH in the calculations, indicates a rather clear-cut difference between the caries-active and caries-immune plaques. The type of curve is probably due to the fact that the pH is a function of the logarithm of a ratio; however, regardless of the cause, it yields a clear-cut difference between the caries-active and caries-immune plaques. If we assume that the maximum safe free acid concentration is 3 mM per kilogram dry weight, then 100 per cent of the caries-immune

plaques fall in this category and 90 per cent of the caries-active cases do not. This concentration occurs at pH 5.5 to 5.4 for the immune cases and 5.6 to 5.7 for the caries-active. In this area the slope of the curve changes precipitously and the free acid increases rapidly at acidities above these values. Thus, it may be said that the critical pH is somewhere below pH 5.7 and somewhere above pH 5.2. On the basis of the free lactic acid, 3 mM per kilogram or below should be safe.

It would seem that the state of the lactate in the dental plaque may be one of the important factors associated with dental caries.

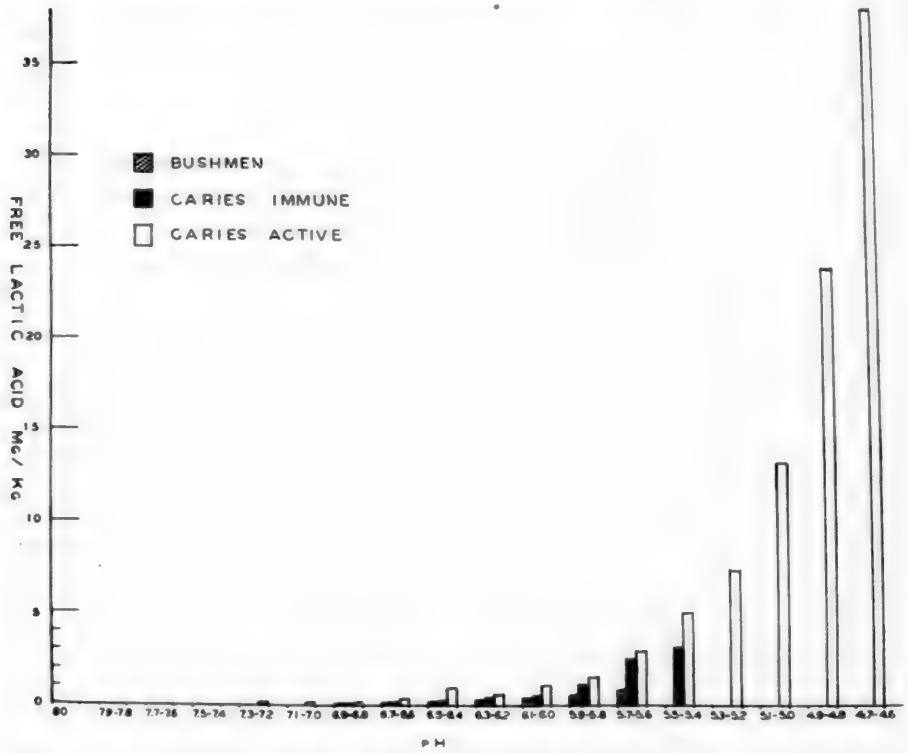


Fig. 3.—The average free lactic acid in dental plaques as calculated from analytic data shown in Fig. 2.

SUMMARY

The pH and lactate content of caries-immune plaques have been determined. A correlation of the pH, total lactate, and free lactic acid in caries-active, caries-immune, and Bushmen plaques has been made. On the basis of the minimum pH and maximum lactate found in caries-immune mouths and Bushmen, it is quite likely that pH values of 5.7 or above and 3 mM per kilogram free lactic acid or less are harmless.

REFERENCES

1. Moore, B. W., Carter, W. J., Dunn, J. K., and Fosdick, L. S.: The Formation of Lactic Acid in Dental Plaques. I. Caries-Active Individuals, *J. D. Res.* **35**: 778, 1956.
2. Clement, A. J., Plotkin, R., and Fosdick, L. S.: The Formation of Lactic Acid in Dental Plaques. II. Oral Conditions of Primitive Bushmen of the Western Kalahari Desert, *J. D. Res.* **35**: 786, 1956.
3. Jay, P., and Arnold, F. A.: Dental Caries and Fluorine, Lancaster, 1946, Science Press Printing Company, p. 46.
4. Fosdick, L. S., Campaigne, E. E., and Fancher, O. E.: Rate of Acid Formation in Carious Areas: The Etiology of Dental Caries, *Ill. D. J.* **10**: 85, 1941.
5. Barker, S. B., and Summerson, W. H.: The Colorimetric Determination of Lactic Acid in Biological Material, *J. Biol. Chem.* **138**: 535, 1941.

THE RELATIVE EFFICIENCY OF TWO SELECTIVE MEDIA FOR THE ISOLATION OF LACTOBACILLUS SPECIES FROM THE MOUTH

G. CHARLTON, M.D.S., AND H. C. SPIES, A.S.T.C., A.R.A.C.I.

Institute of Dental Research, United Dental Hospital, Sydney, Australia

TOMATO-PEPTONE agar medium[1] has been widely used for the selection and enumeration of oral lactobacilli in a laboratory method for assessing susceptibility to dental caries. The modification of the Kulp-Hadley medium[1] used in this laboratory is not completely selective and difficulty is experienced at times in differentiating between lactobacilli and some aciduric, acidogenic staphylococci. For routine purposes it is desirable to have a more selective medium.

This investigation was undertaken to establish (a) the relative selectivity (with respect to the lactobacillus) of Rogosa S.L. medium[2, 3] and tomato-peptone agar and (b) the relative abilities of these 2 media to support the growth of lactobacilli from the saliva.

MATERIALS AND METHODS

(a) Rogosa S.L. agar, containing sorbitan monooleate (Tween 80) (Atlas Powder Co., Delaware, U. S. A.).

(b) Tomato-peptone agar (T.P.A.). This was a modified Kulp-Hadley medium prepared as follows:

(1) Peptonized milk, Difco	10 Gm.
Neopeptone, Difco	10 Gm.
Tomato juice, prepared from whole canned fruit	600 ml.

pH adjusted to 7.0 with NaOH, then lowered to 5.0 with lactic acid.

(2) Agar (Davis, New Zealand)	15 Gm.
Distilled water	400 ml.

Dissolved by autoclaving at 15 lb. pressure for 15 minutes.

After mixing (1) and (2) the medium is dispensed and autoclaved at 10 lb. pressure for 10 minutes.

Media (a) and (b) were freshly prepared and dispensed in 15 ml. amounts in 9 cm. Petri plates.

(c) 1 per cent glucose beef heart infusion broth, adjusted to pH 7.0.

(d) Saliva. Five-minute, paraffin-stimulated samples from patients attending the United Dental Hospital, were allowed to stand for 30 minutes.

After thoroughly mixing the samples of saliva by shaking, serial dilutions in 1 per cent peptone water of each sample of saliva (1/1, 1/10, 1/100, 1/1000)

Received for publication March 17, 1955.

were plated (a) on tomato peptone agar, with the aid of glass spreaders, and (b) in Rogosa S.L. agar, by the pour plate technic. All plates were incubated aerobically at 37° C. for 4 days.

Examination of the plates was made independently by each of 2 observers. Colonies of all morphologically distinctive types were counted. Where possible, plates containing 25 to 250 colonies were chosen for counting. Representatives of each colony type were stained by Gram's method and subcultured into glucose broth to be incubated aerobically.

The glucose broth cultures were examined for: motility (after 24 hours' growth), growth characteristics (at 24-hour intervals), morphology (by Gram stain), gas production (Durham tubes), acid production (Beckman model G potentiometer, glass electrode), and catalase production (1 ml. fresh H_2O_2 10 vols. per 5 ml. culture medium) after 72 hours' incubation.

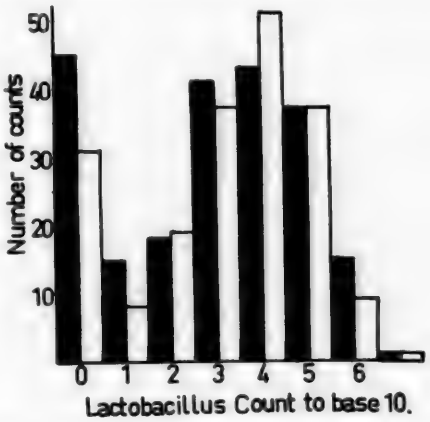


Fig. 1.—Distribution histogram showing the number of saliva samples in which the lactobacillus count fell into certain numerical ranges.
Rogosa S. L. medium.
Tomato-peptone agar.

Those organisms, isolated on these media, which appeared in subculture to be acid-producing, nonsporing, nonmotile, catalase-negative, Gram-positive rods, were considered to be lactobacilli. They were divided into heterofermentative and homofermentative groups by their ability or failure to produce gas. The number of lactobacilli per milliliter of saliva was calculated and corrected for standardization on the basis of a 10 ml. sample.

RESULTS AND DISCUSSION

Two hundred individual saliva samples were examined and from these the following numbers of organisms were isolated:

Rogosa: 354 isolates, of which 302 were homofermentative lactobacilli, 50 heterofermentative lactobacilli, 1 aciduric, acidogenic staphylococcus, 1 yeast-like organism.

T.P.A.: 557 isolates, of which 383 were homofermentative lactobacilli, 68 heterofermentative lactobacilli, 54 aciduric, acidogenic staphylococci, 51 yeast-like organisms, 1 streptococcus.

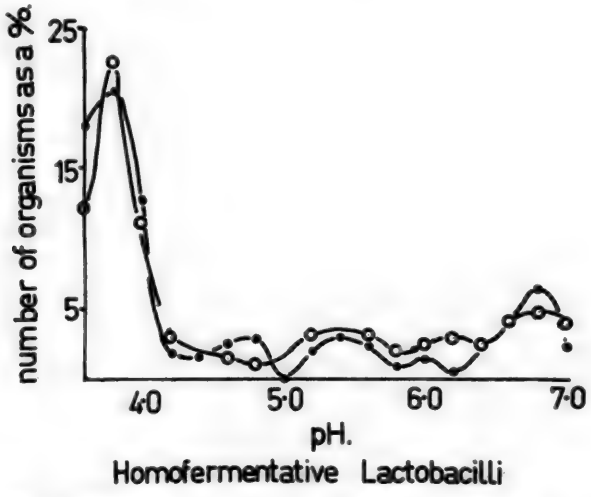


Fig. 2.—The number of heterofermentative lactobacilli, expressed as a percentage of the total number of lactobacilli, isolated on each medium and plotted against the final pH produced after 3 days' incubation in 1 per cent glucose broth.
—●—● Rogosa S. L. medium.
—○—○ Tomato-peptone agar.

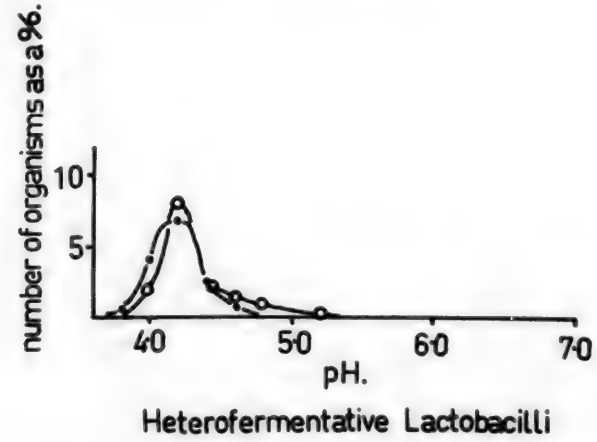


Fig. 3.—The number of homofermentative lactobacilli, expressed as a percentage of the total number of lactobacilli, isolated on each medium and plotted against the final pH produced after 3 days' incubation in 1 per cent glucose broth.
—●—● Rogosa S. L. medium.
—○—○ Tomato-peptone agar.

On those plates where colonial development was not obscured by the presence of large numbers of colonies of lactobacilli, the staphylococci occurred only in small numbers (27 times in the 1/1 dilutions, 17 times in the 1/10 and 4

times in the 1/100). On most plates they could be distinguished by their colonial form. The cocci isolated on the T.P.A. medium invariably lowered the pH to the range 4.2 to 4.5.

The work of Permar, Kitchin and Robinson[4] has shown that the numbers of lactobacilli recovered in a series of counts, each made from a single specimen of saliva, may vary over a wide range.

Examination of the counts for each saliva sample on the 2 media indicated that any discrepancies could be assumed to be sampling errors and were of such an order that they did not influence the assessment of caries susceptibility. If the 2 media gave similar results, distribution curves of the lactobacillus counts would be alike. Fig. 1 shows the number of saliva samples in which the lactobacillus count fell into similar numerical ranges.

The recovery of different types of lactobacilli (homofermentative and heterofermentative) from each medium appeared to be similar (Figs. 2 and 3) as also the physiologic ability of these types to increase the hydrogen ion concentration in 1 per cent glucose broth.

Technically, the pale color of S.L. medium lends itself well to counting, in that it allows easy detection of the colonies which, on the whole, develop well and are usually from 2.0 to 4.0 mm. in diameter. However colonies as small as 0.4 mm. in diameter occur which could be confused with aggregates of debris in the medium. Difficulty was experienced with the Rogosa S.L. medium in differentiating between types of lactobacilli on a colony form basis, because the majority of colony forms are similar, being biconvex discs.

Contaminating moulds did not occur on either medium during these experiments but experience in this laboratory suggests that, in laboratories where contamination is a problem, the S.L. medium would be the more satisfactory.

SUMMARY

Two selective media, Rogosa S.L. agar and tomato-peptone agar, have been examined with respect to their relative efficiency for the isolation of lactobacillus species from the mouth.

From this work it may be concluded that Rogosa S.L. agar is more selective for oral lactobacilli than the tomato-peptone agar and that, as similar numbers of lactobacilli can be recovered from saliva samples, it is a suitable medium for use in routine lactobacillus counts.

REFERENCES

1. Hadley, Faith P.: A Quantitative Method for Estimating *Bacillus acidophilus* in Saliva, *J. D. Res.* **13**: 415-428, 1933.
2. Rogosa, M., Mitchell, Joyce A., and Wiseman, R. F.: A Selective Medium for the Isolation and Enumeration of Oral Lactobacilli, *J. D. Res.* **30**: 682-689, 1951.
3. Rogosa, M., Wiseman, R. F., Mitchell, Joyce A., and Disraley, M. N.: Species Differentiation of Oral Lactobacilli from Man Including Descriptions of *Lactobacillus salivarius* nov spec and *Lactobacillus cellobiosus* nov spec, *J. Bact.* **65**: 681-699, 1953.
4. Permar, D., Kitchin, P. C., and Robinson, H. B. G.: Variations in Counts of Lactobacilli made from Single Specimens of Saliva, *J. D. Res.* **25**: 475-486, 1946.

OBSERVATIONS OF HUNTER-SCHREGER BANDS

J. F. MORTELL, JR., D.D.S., AND F. A. PEYTON, D.Sc.

(School of Dentistry, University of Michigan, Ann Arbor, Mich.)

DURING the process of studying the physical damage and traumatic effects of various instruments on tooth structure, it became apparent that some consideration should be given to the Hunter-Schreger band formation as it exists in enamel structure. When examined by reflected light, these bands appear as alternating light and dark areas in the enamel portion of a longitudinal ground-tooth section, starting at, or near, the dentino-enamel junction and passing outward toward the enamel periphery.

The Hunter-Schreger bands have been described generally as an optical manifestation of the direction in which the enamel rods have been sectioned relative to their axis. Despite many detailed studies of these bands, their significance, physical structure, and origin still remain obscure. It was because of the need for a better understanding of the physical arrangement of the enamel rods in human tooth enamel that this examination of the Hunter-Schreger bands was undertaken. This report will describe the results obtained when a method for examining Hunter-Schreger bands by means of a shadowed replica technic and shadowed tooth sections is employed. This method differs somewhat from the conventional methods which have been used in the past, that made use of examination of etched and unetched longitudinal ground sections with reflected light, or thin sections by transmitted light, and partially decalcified stained sections.

REVIEW OF LITERATURE

These band formations in tooth enamel were first described by Schreger in 1800,[6] although they apparently were observed as early as 1770 by Hunter.[8]

A microscopic examination of the tooth usually reveals a grouping of enamel rods into bundles or bands that present different orientations to the longitudinal section of the tooth. In some groups or bundles the rods appear to have been sectioned longitudinally, while in adjacent groups the rods appear to have been cut transversely or obliquely. Preiswerk[15, 16] gave the name parazone to the groups of rods that are cut longitudinally, and diazone to those that are cut transversely. The relationship of the parazones and the diazones has furnished the basis for many of the explanations of the phenomenon of Hunter-Schreger bands in enamel.[2-5, 7, 9-11, 13, 14, 17-20, 23-26]

This study was supported by a grant to the University of Michigan, School of Dentistry, from the U. S. Air Force, School of Aviation Medicine, Contract AF 18(600)-427.

Presented at the meeting of the Dental Materials Group, I.A.D.R., Chicago, March 18-20, 1955.

Received for publication April 18, 1955.

Various views regarding the origin and significance of the banded formation have been given. Some investigators explain the appearance of the bands as being due to a difference in density of calcification of the alternating zones.[1, 6, 22] Others report the bands to be due to a difference in organic content of the adjacent zones of the enamel.[21] At least one report describes the bands as an optical phenomenon with but little histologic significance,[12] while another investigator considers the regular occurrence of the bands to indicate a definite anatomic arrangement of the structural units of the enamel.[2]

MATERIALS AND METHODS

For this study, freshly extracted teeth, both with and without fillings, which were kept immersed in water following their extraction, were ground various distances pulpally from the mesial, distal, lingual, or buccal aspect of the crown. When the required depth was reached, the surface was polished serially with 0 through 4/0 metallographic polishing paper. Some polished specimens were examined by reflected light without further treatment, while others were etched for a period of 5 to 25 seconds with 0.1 normal hydrochloric acid and dried. A portion of the etched specimens was shadowed with copper in vacuum, while others were coated with 2 or 3 applications of a dilute collodion solution. After the collodion film was dry, the teeth were returned to complete submersion in water for 24 hours, to facilitate the stripping off of the large collodion replicas without tearing. The collodion replicas were then placed in a drop of water on a glass microscope slide and pressed flat, when necessary, with a cover glass. After thorough drying the replicas were shadowed with copper in vacuum, using the technic essentially as reported by Williams and Wyckoff.[29] A total of approximately 650 replicas and sections have been shadowed and examined.

In addition to the above specimens, 40 tooth surfaces were polished through 4/0 polishing paper and replicas were taken of the surfaces both before and after etching. Another group of 40 tooth surfaces were polished in the same manner, after which 20 were etched and 20 were left unetched, and subsequently both groups of teeth were dried and shadowed directly. A fourth group of teeth were cut into thin sections by rotary carborundum disks, after which they were partially decalcified by immersion in 1 normal hydrochloric acid for periods of 1 to 5 minutes, then stained with hematoxylin and examined by transmitted light.

MICROSCOPIC OBSERVATIONS

In Fig. 1 is represented a typical band structure as observed by microscopic examination of an unetched polished section of a molar tooth when examined by reflected light. This tooth was not shadowed with copper, and even though in certain areas the bands can be observed to extend to the enamel periphery, the details of the bands are not sharp and their structure is not clear. This appearance is typically characteristic of polished, unetched sections of teeth.

Repeated efforts failed to reveal any evidence of Hunter-Schreger bands when a collodion replica was made of a polished but unetched enamel surface.

Even though it was possible to observe the boundary of a restoration, and on occasion the dentin-enamel boundary could be distinguished, there was no evidence of difference in surface structure due to band formation. Similar results were observed when the tooth was polished through 4/0 paper and shadowed directly with copper before being etched. Beyond observing the difference in surface texture between enamel and dentin, no structural details were evident. Particularly, no Hunter-Schreger bands were demonstrated by shadowing the polished, unetched tooth section. The checked and cracked enamel was observed which probably was the result of dehydration of the tooth that was necessary for the shadowing operation.

Fig. 1.—Polished but unetched tooth section as observed by reflected light.

When the tooth surface was etched for 15 seconds with 0.1 normal hydrochloric acid, the band structure began to be revealed, as shown in Fig. 2, by a shadowed replica. Shown at only 10 diameters magnification and perhaps an incomplete etch, evidence of the band formation is apparent, extending outward to the enamel periphery. The outline of the metal restoration also can be distinguished.

The etched tooth surface from which the replica was produced for Fig. 2 subsequently was shadowed by copper in vacuum, following the stripping of the collodion replica. The structure revealed by the shadowed tooth is shown in Fig. 3, at 10 diameters magnification, and is a reverse of the shadowed replica shown in Fig. 2. While the band details are not pronounced in either the shadowed tooth (Fig. 3) or the replica (Fig. 2), it is believed that this lack of detail is due to incomplete etching of the tooth, but either the tooth or replica can be used to reveal the band detail.

In Fig. 4 is shown a shadowed replica of a ground tooth surface, etched for 20 seconds, and photographed at the low magnification of 10 diameters. The path of the Hunter-Schreger bands passing outward from the dentino-enamel junction is demonstrated, with the bands appearing to extend to the periphery of the enamel. Numerous examples of teeth revealed the same type of band structure. In particular, they demonstrate clearly a concentric arrangement of the bands in the cuspal areas which has not been widely reported in the past, although at least four investigators have suggested this formation.[2, 23, 27, 28]

Fig. 2.

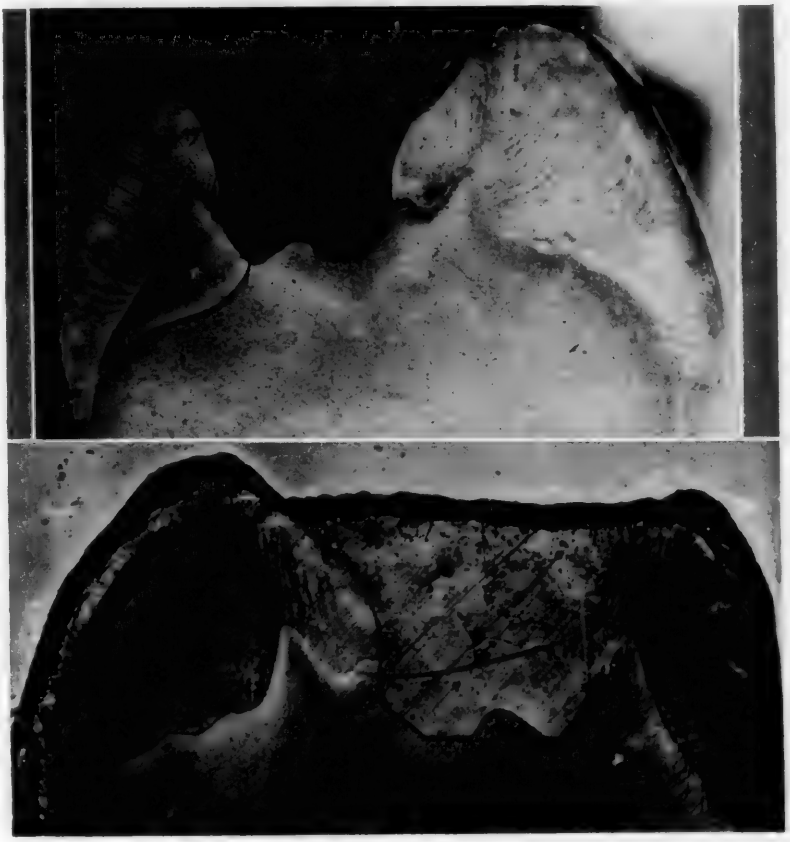


Fig. 3.

Fig. 2.—Replica of polished section, etched 15 seconds with 0.1 normal hydrochloric acid.

Fig. 3.—Shadowed tooth from which replica was taken for Fig. 2.

The bands represented in Fig. 5 were observed from a polished tooth surface that was etched for 20 seconds with 0.1 normal acid before being shadowed with copper. The longer etch seems to have developed a more pro-

nounced band structure, revealing the concentric arrangement in the cuspal area, and the extension of the bands to the enamel periphery. The outline of a metal restoration is also clearly shown in Fig. 5.

The enamel structure shown in Fig. 6 is that of a shadowed replica of a polished tooth section, etched for 20 seconds with 0.1 normal hydrochloric acid, and photographed at a magnification of approximately 100 diameters.

Fig. 4.

Fig. 5.

Fig. 4.—Replica of polished section, etched 20 seconds with 0.1 normal hydrochloric acid, showing concentric arrangement of bands in cusp area.

Fig. 5.—Replica of polished section containing metal restoration and showing band formation in cusp area.

The area represents the boundary between a metal restoration and enamel. The characteristic light and dark Hunter-Schreger bands can be observed. At this magnification there is evidence of rod ends in the enamel, but a change in rod direction in the alternate bands is not apparent. This was a consistent observa-

tion of the rod direction near the periphery of the enamel, even though the band formation was routinely found in the peripheral third of the enamel structure.

In Fig. 7 is shown the structure resulting from a shadowed replica of a polished enamel surface that was etched for 10 seconds with 0.5 normal hydrochloric acid, and photographed at a magnification of 400 times. Greater detail is registered as a result of the more pronounced etch and the higher magnification. The different orientation of the bands, corresponding to the parazones and diazones, is also evident in this illustration. At a higher magnification of approximately 700, the same area develops the appearance shown in Fig. 8.

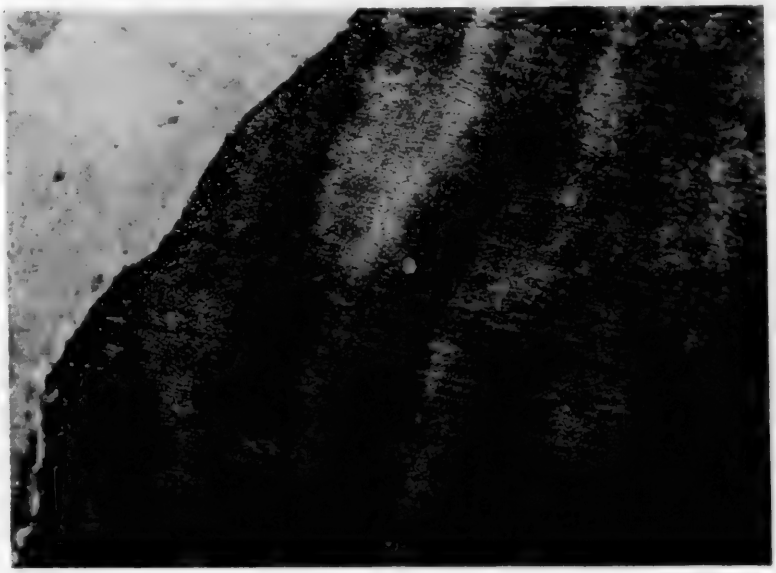


Fig. 6.—Enamel structure at margin of restoration showing band formation (×100).

Fig. 9 shows a thin section, partially decalcified in 1 normal hydrochloric acid for five minutes and stained with hematoxylin. The bands are not demonstrated in the outer one-third of the enamel and rarely in cuspal areas when this method is used. Shown in Fig. 10 is a high magnification of an area from Fig. 9. Change in rod direction corresponding to parazones and diazones is demonstrated.

DISCUSSION OF RESULTS

Accepting the fact that this does not represent an exhaustive study of the Hunter-Schreger band formation, it appears, however, that the application of the replica method offers promise in refining our knowledge of histologic structure of dentin and enamel. Throughout the study it has been observed that the replica method is more sensitive for the study of Hunter-Schreger bands than hematoxylin staining. The response to acid varied considerably in different

Fig. 7.

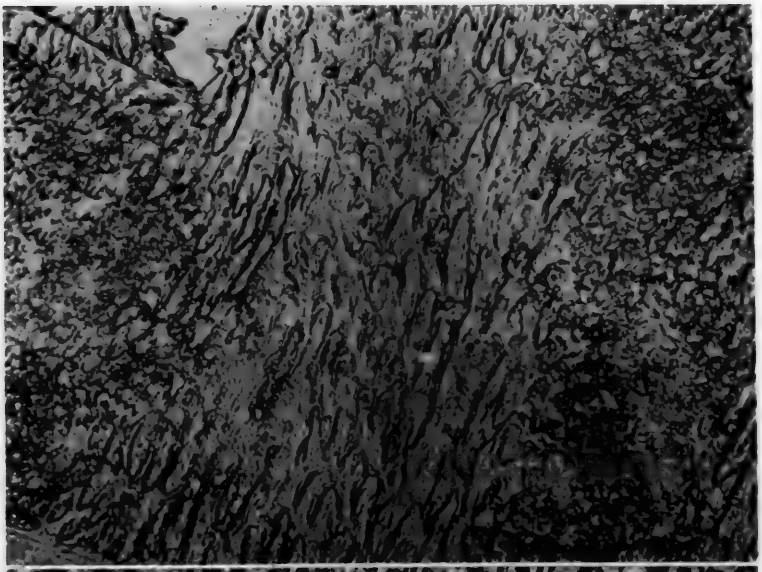


Fig. 8.

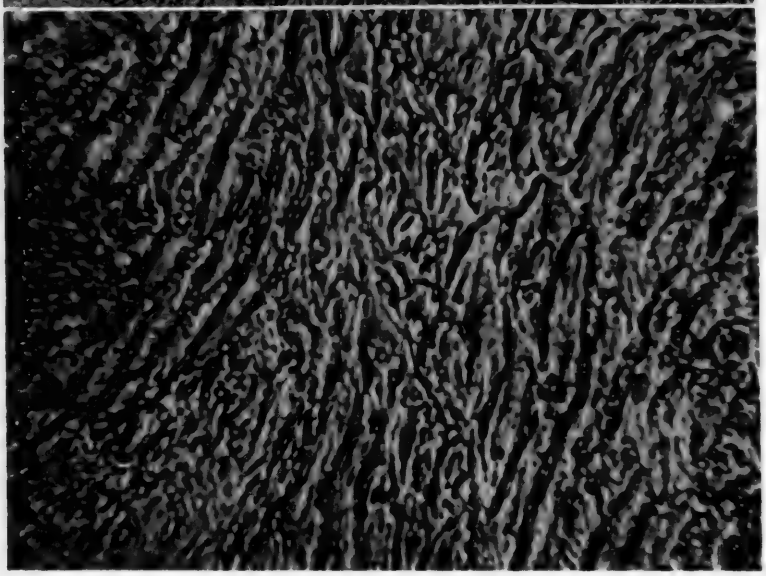


Fig. 7.—Replica section of enamel etched 10 seconds with 0.5 normal hydrochloric acid (approximately ×400).

Fig. 8.—Higher magnification of portion of replica shown in Fig. 7 (approximately ×700).

areas of the same tooth, as has been observed by others, but not in any particular pattern. On some surfaces the response to the etching varied to the extreme of having the appearance of an unfathomable surface in the replica in so far as the detail structure of the enamel was concerned, yet the Hunter-Schreger bands were recorded consistently.

Fig. 9.

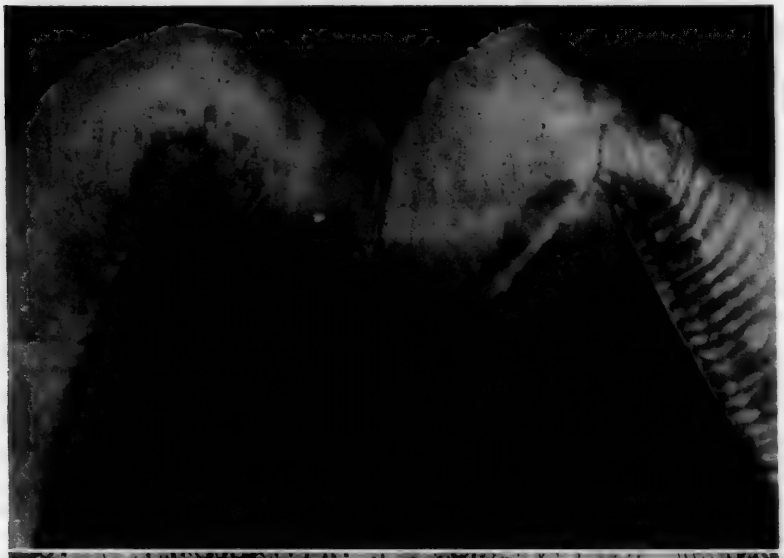


Fig. 10.

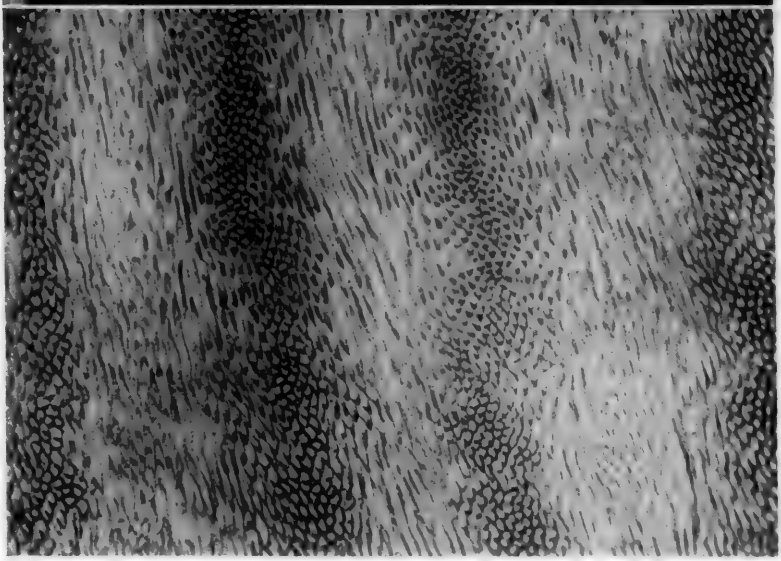


Fig. 9.—Thin section of tooth partially decalcified and stained with hematoxylin.
Fig. 10.—Higher magnification of portion of section shown in Fig. 9.

While areas on replicas were readily found where Hunter-Schreger bands correspond to parazones and diazones, many times these bands were observed in areas where no change in rod direction was apparent. Thus, it would appear that an explanation of the Hunter-Schreger bands entirely on the basis of changes in direction of the rods may be an oversimplification of the process. Some additional study of rod orientation should be useful.

In general, the dark bands in the replicas examined displayed more detailed structure than the light bands. The contrast between the light and dark bands in regard to structure, discernible within the rod sheath, was striking. The structureless appearance within the rod sheaths, regardless of the direction sectioned, was characteristic of the light bands. Staz reported what may be a related observation, that "diazones are more heavily stained than the parazones."[27]

All attempts to record Hunter-Schreger bands in replicas of polished but unetched surfaces of teeth were unsuccessful. Each of the surfaces so studied was etched subsequently with hydrochloric acid, and the shadowed replicas then obtained revealed Hunter-Schreger bands. The shadowing of the surfaces of the teeth directly yielded a similar result, namely, no evidence of the bands until the surfaces were etched. This might be taken as some evidence that the band formation is produced by the physical structure within the enamel and is not entirely optical in nature.

It would appear that the depth of etching alternated somewhat uniformly to demarcate the Hunter-Schreger bands, with the dark bands in the replica indicating areas of relatively deeper etching than the light bands. The dark bands of the replica are interpreted as areas of high content of organic matrix.

It can be demonstrated in some replicas that the optical phenomenon of changing of bands from light to dark, and vice versa, can be produced in the conventional manner of changing the light source by 180°, and also more simply by a small change in the mirror when viewing the bands by transmitted light. Numerous other optical effects could be developed. Further study to explain the optical characteristics of the Hunter-Schreger bands in replicas is desirable.

Three morphologic characteristics of Hunter-Schreger bands seem to be evident from this study. (a) The bands are revealed to extend from the dentino-enamel junction to the periphery of the enamel in many teeth when examined by the shadowed replica method. (b) The bands form concentric arcs in the cuspal areas, as a characteristic formation. (c) The Hunter-Schreger bands may be observed in areas of the enamel where change in rod direction is not apparent.

CONCLUSIONS

1. The Hunter-Schreger bands are a consistent finding in shadowed replicas of etched surfaces of enamel.

2. The shadowed replica offers a method for study of details of Hunter-Schreger bands not previously described.

3. Hunter-Schreger bands do not always start at the dentino-enamel junction, but may exist as concentric arcs in cuspal areas.

4. The detection of typical Hunter-Schreger bands in shadowed replicas of etched tooth surfaces suggests that the mineralization of the tooth is such that alternating bands of greater and lesser resistance to etching exist.

5. The Hunter-Schreger bands can be followed from the dentino-enamel junction to the periphery of the enamel by the use of shadowed replicas.

REFERENCES

1. Berke, J. D.: Studies in the Histology and Pathology of Human Enamel, *D. Cosmos* **78**: 700-710, 1936.
2. Beust, T. B.: Dental Histology and Embryology, Philadelphia, 1934, W. B. Saunders Co.
3. Caush, D. E.: Is There Uncalcified Tissue in the Enamel? *D. Cosmos* **47**: 165-77, 1905.
4. Churchill, H. R.: Meyer's Histology and Histogenesis of the Human Teeth, Philadelphia, 1935, J. B. Lippincott Co., pp. 23-27.
5. Gustafson, G.: The Structure of Human Dental Enamel, Suppl. *Odontologisk Tidskrift* **53**: 65-72, 1945.
6. Hollander, F., Bodecker, C. F., Applebaum, E., and Saper, E.: A Study of the Bands of Schreger by Histological and Grenz-Ray Method, *D. Cosmos* **77**: 12-20, 1935.
7. Hopewell-Smith, A.: The Normal and Pathological Histology of the Mouth, Philadelphia, 1918, The Blakiston Co., Vol. 1, pp. 37-39.
8. Hunter, J.: The Natural History of the Human Teeth, London, 1771.
9. Kollman, J.: Dentin, Enamel, Cement, pp. 354-401. (In Siebold and Kolliker, editors, Zeit. f. Wissenschaftliche Zoologie, Leipzig, 1873, Wilhelm Engelmann.)
10. Kronfeld, R.: Dental Histology and Comparative Dental Anatomy, Philadelphia, 1937, Lea & Febiger, p. 20.
11. Leicester, H. M.: Biochemistry of the Teeth, St. Louis, 1949, The C. V. Mosby Co., p. 122.
12. Mummery, J. H.: The Microscopic Anatomy of the Teeth, London, 1919, Frowde, Hoddler & Stoughton, p. 77.
13. Noyes, R. B.: A Textbook of Oral Histology and Embryology, ed. 2, Philadelphia, 1921, Lea & Febiger, p. 73.
14. Orban, B.: Dental Histology and Embryology, ed. 2, Philadelphia, 1929, The Blakiston Co., p. 14.
15. Preiswerk, G.: Beiträge zur Kenntniss der Schmelzstruktur bei Saugethieren, Basel, 1895, pp. 87-92.
16. *Idem:* Lehrbuch und Atlas der Zahnheilkunde, München, 1903, pp. 61-65.
17. Saunders, J. B. de C. M., Nuckolls, J., and Frisbie, H. E.: *Am. Coll. Dent. J.* **9**: 107-136, 1942.
18. Schour, I.: Noyes' Oral Histology and Embryology, ed. 7, Philadelphia, 1953, Lea & Febiger, p. 106.
19. Schour, I., and Noyes, H. J.: Noyes' Textbook of Oral Histology and Embryology, ed. 5, Philadelphia, 1938, Lea & Febiger, p. 108.
20. Scott, D. B., and Wyckoff, R. W. G.: Shadowed Replicas of Group Sections Through Teeth, *Pub. Health Rep.* **62**: 442-45, 1947.
21. Sognnaes, R. F.: The Organic Elements of the Enamel, II. The Organic Framework of the Internal Part of the Enamel With Special Regard to the Organic Basis for the So-called Tufts and Schreger's Bands, *J. D. Res.* **28**: 549-57, 1949.
22. Staz, J.: Dental Caries in South Africa, *South African J. M. Sc.* **3**: 1-63, 1938 (Suppl.) pp. 37-38.
23. *Idem:* Bands of Schreger, *J. D. Res.* **25**: 373-80, 1946.
24. Tims, H. W., and Henry, D. B.: Tomes' Dental Anatomy, New York, 1923, The Macmillan Co., p. 56.
25. Tomes, C. S.: A Manual of Dental Anatomy. Philadelphia, 1898, The Blakiston Co., p. 27.
26. Von Ebner, V.: Histologie der Zähne mit Einschluss der Histogenese, pp. 209-262. (In Scheff, Julius, editor: Handbuch der Zahnheilkunde, Wien, 1891, Alfred Holder.)
27. *Idem:* Kolliker's Handbuch der Fewelehre des Menschen, 6th revision, Leipzig, 1902, Wilhelm Engelmann, pp. 88-89.
28. Walkhoff, O.: Die normale Histologie menschlicher Zähne, Leipzig, 1901, Felix.
29. Williams, R. C., and Wyckoff, R. W. G.: Applications of Metal Shadow-Casting to Microscopy. *J. App. Phys.* **17**: 23-33, 1946.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Annual Meeting—St. Louis, Mo.

March 22, 23, 24, 1956

EXECUTIVE PROCEEDINGS

A. *COUNCIL ACTIVITIES*

During the year between meetings the Council nominated candidates for the general officers of the Association. The names of these candidates appeared on the official ballot of the Association. A membership committee (Sognnaes, McClure, Manly), on behalf of the Council, reviewed the credentials of persons nominated to membership during the year and reported to the Council.

Council Meetings, March 23 and 24, 1956

The following persons were present at Council meetings: Arnim, Arnold, Bibby, Blayney, Boyle, Burrill, Coy, Crawford, Dean, Emslie, Grossman, Hall, Hill, Hodge, Kerr, Kitchin, Kreshover, Lefkowitz, Levy, Manly, Minoguchi, Myers, Orland, Robinson, Rosebury, Scott, Shafer, Simon, Sognnaes, Swanson, Thomas, and Volker.

The dues for the Association were set at the same level as in the preceding year, as follows:

> For members residing in North America, $10.00, of which $3.00 is appropriated to the general fund of the Association and $7.00 for subscription to the Journal of Dental Research.
>
> For members residing outside North America, $3.00 dues. The fee for subscription to the Journal of Dental Research, $7.00, is payable only if the member wishes to receive the Journal. The officers are authorized to reach a compromise on dues for any individual living outside North America.

To assist in the nomination of general officers of the Association, the Council directed that a nominating committee be created, to consist of the five latest past presidents of the Association. The committee will select nominees whose names will then be submitted for action by the Council. The nominating committee for the 1956-57 Association year consists of Boyle (Chairman), Paffenbarger, Arnold, Hine and Fosdick.

The Council directed that an Archives Committee be appointed to collect material of historical interest, record and preserve it. The president appointed as the committee, Orland (Chairman), Hatton and Levy. Documents and information of particular historical interest to the Association should be sent to Dr. Frank Orland.

The Publicity Committee (Pearlman, Kreshover, Williams) submitted, and the Council approved, a resolution that a permanent publicity committee be constituted, to consist of three members holding office for three years each, with terms of office staggered, to be appointed by the president.

The Council directed that the Secretary notify members of the Association as follows:

NOMINATIONS TO MEMBERSHIP IN THIS ASSOCIATION MUST BE IN THE HANDS OF THE SECRETARY OR THE MEMBERSHIP COMMITTEE by February 15, 1957, if they are to be considered at the March, 1957, meeting. Completed nomination forms with supporting material should be sent to the Assistant Secretary-Treasurer, Dan Y. Burrill, 129 E. Broadway, Louisville 2, Ky., or to the chairman of the membership committee, Dr. R. S. Manly, 136 Harrison Avenue, Boston 15, Mass.

B. *EXECUTIVE SESSIONS*

1. March 22, 1956. 8:00 P.M.

A quorum being present, the meeting was opened by the President, Dr. Boyle, with appropriate remarks. Greetings from Dr. Gies and Dr. Hatton were extended to the Association, with best wishes for a successful meeting.

The minutes of the 33rd general meeting, March, 1955, were approved as published in the JOURNAL OF DENTAL RESEARCH for October, 1955.

Tellers were appointed to count the votes for general officers of the Association and to report at the final executive session.

The Assistant Secretary-Treasurer presented the audited report on the finances of the offices of Secretary-Treasurer and Assistant Secretary-Treasurer for the 1955 calendar year. The report was accepted and approved and is printed in a later section. It was pointed out that while the report showed an increase in the Association bank balance, the increase was due to early collections of dues, subscription fees and registration fees for the ensuing year and to delay in making certain payments, so that there actually is no improvement in the financial position of the Association. It was noted that receipts from the 1955 meeting did not pay the costs of the meeting, leaving a deficit of $462.11 which was paid out of the general fund of the Association. Also, because of failure of members to pay dues and subscription fees on time, the Association had paid out for subscriptions $182.00 more than was received. The Association has sufficient reserve for current needs, but in the future it will be necessary to increase the dues or the registration fee for the meeting, or both, in order to keep the Association solvent. The Association now has 808 members; 671 members reside in North America. Of these, 97 are dues remitted members, leaving 574 dues paying members in North America. Outside of North America there are 137 members, 87 of whom have paid dues. On the basis of the above membership plus the new members, and attempting to foresee the probable necessary expenditures, we anticipate a deficit for the 1956 calendar year.

Dr. Hamilton Robinson reported that funds obtained from the Supporting Associates of the JOURNAL paid for 64 extra pages in the JOURNAL during the year, but that publication of papers was still delayed fourteen months after receipt.

Dr. Blayney, as chairman of the committee on Reorganization and Administration, presented a revision of the Constitution and By-laws of the Association, as a petition for amendment of the constitution as provided by the constitution. Dr. Boyle accepted the revision as a petition and appointed the Committee on Reorganization and Administration to continue as a committee to carry through the procedures necessary to bring the amendment to a vote by the Association.

The petition for amendment of the constitution, consisting of one copy of the revision proposed by the committee plus some changes made at the time of the meeting, all of which were specifically approved by all signers, was signed by the following members of the Association:

> Ned B. Williams, Frank J. Orland, Iden N. Hill, Hamilton Robinson, J. R. Blayney, H. Trendley Dean, Paul E. Boyle, Reidar F. Sognnaes and Dan. Y. Burrill.

On behalf of a budget committee appointed at the 1956 meeting, Dr. Burrill proposed a budget for the offices of the Secretary-Treasurer and Assistant Secretary-Treasurer for the 1956-57 Association year. The budget was approved and is printed in a later section.

Dr. Williams reported that the committee on meeting places had conferred with Dr. McCrea as representative of the American Association of Dental Schools and that the following meeting places had been designated for the two Associations:

> 1957—Atlantic City, N. J.
> 1958—Detroit, Mich.
> 1959—San Francisco, Calif.
> 1960—Chicago or French Lick, Ind.

1961—Boston, Philadelphia or Atlantic City
1962—St. Louis, Chicago or French Lick
1963—Pittsburgh or Cleveland

On behalf of the local arrangements committee, Dr. Brandhorst welcomed the Association to St. Louis.

The meeting adjourned, 9:00 P.M.

2. Final Executive Session, March 24, 1956. 5:00 P.M.

A quorum being present the session was convened as a meeting of the North American Division of the Association. The appointment of S. Wah Leung as representative to the American Association for the Advancement of Science was approved. Dr. Volker presented resolutions urging the provision of Federal money for the support of dental research and urging provision of a new building for the National Institute of Dental Research. The resolutions were adopted. Dr. Hill presented, and the Division adopted a resolution thanking the American Dental Association personnel, especially Dr. Harold Hillenbrand, for efforts to increase the Federal support of dental research.

The meeting of the North American Division was adjourned and the session immediately reconvened as a meeting of the Association.

Dr. Boyle reported the action of the Council in setting dues for 1957.

A budget for the publications committee was approved. The budget is printed in a later section. Dr. Burrill requested and, by vote, was granted authority to expend Association funds for the support of the JOURNAL at the discretion of the general officers of the Association, provided such expenditure for the JOURNAL does not place the Association in an unsound financial condition.

The Lincoln Bank and Trust Co., Louisville, Ky., and the Evanston State Bank and Trust Co., Evanston, Ill., were authorized as repositories of general Association funds. The Indiana National Bank, Indianapolis, Ind., was approved as repository of funds for the JOURNAL of the Association. The Association approved disbursement of Association Journal funds from the Indiana National Bank by checks signed by either J. Frank Hall or Hamilton B. G. Robinson, and countersigned by either Joseph F. Volker or Reidar F. Sognnaes.

The thanks of the Association were officially expressed to the University of Louisville and the University of Louisville Print Shop and to Mrs. Dunbar and others at the print shop, for their assistance in preparing the preprinted abstracts of papers on the program; to the students from the dental schools of Washington University and St. Louis University for their assistance at the meeting; and to the persons comprising the several committees which functioned to make the meeting a success.

Dr. Sognnaes reported for the membership committee and the Council that nominations of 67 persons had been approved for election to membership. By unanimous vote the secretary was instructed to cast a single ballot on behalf of the Association for their election to membership. The names of the persons elected appear in a later section.

By unanimous vote of the Association the annual award for the best novice presentation at future meetings was designated the Edward H. Hatton Award, to be presented yearly under the rules previously applying to the novice award.

A list of members who had died during the preceding year was read by the Secretary. The members stood silently in respect for their memory.

Dr. Orland reported for the tellers the election of general officers of the Association for the 1956-57 Association year as listed in Part C of the Supplement.

The newly elected officers were called to the platform and declared duly installed. Dr. Volker gave his presidential address on "The Life of Magitot."

The meeting adjourned sine die.

The annual banquet was held Friday evening, March 23, 1956. Dr. E. V. Cowdry gave the main address on the subject "The Antisocial Behavior of Cancer Cells."

The Novice Award was presented to Dr. Barry G. Miller for his paper on "The Influence of Vascularity and Innervation on Resorption of Deciduous Teeth and Eruption of Permanent Teeth."

The Souder Award was presented to Dr. Eugene W. Skinner for his contributions to the science of Dental Materials.

SUPPLEMENT TO PROCEEDINGS

A. FINANCIAL STATEMENTS FOR YEAR ENDED DECEMBER 31, 1955.

1. Evanston, Ill.—Office of Secretary-Treasurer

Endowment Fund		
2 U.S. Govt. bonds (face value)		$1,600.00
1 U.S. Govt. bond—gift of Boston section (face value)		50.00
Balance in Evanston State Bank and Trust Co.		152.34
		$1,802.34
Accrued income for 1955		42.65
Receipts	None	
Disbursements	Clerical	27.95
Gratuity—J. F. Hall		250.00
Gratuity—H. Robinson		250.00
		$ 527.95
Balance in Evanston State Bank and Trust Co.		346.16

2. Louisville, Ky.—Office of Asst. Secretary-Treasurer

Fund balance, January 1, 1955			$4,332.36
Receipts:			
Subscriptions		4,809.97	
Dues		2,204.23	
Registration Fees		1,218.00	
Sale of Banquet Tickets		935.00	
Miscellaneous		116.14	9,283.34
Total			$13,615.70
Disbursements:			
Printing and Distributing JOURNAL (C. V. Mosby Co.)		4,683.00	
Mailing Operations, Postage and Printing		317.69	
Gratuity to Assistant Secretary-Treasurer		500.00	
Annual Meeting Expenses—			
Morrison Hotel	1,598.63		
Printing Programs, Badges, etc.	405.56		
Speaker and Novice Award	200.00		
Cost of Abstracts	361.92	2,566.11	
Miscellaneous		130.39	8,197.19
Fund Balance—December 31, 1955			$5,418.51
Bank Reconcilement			
Cash on Deposit—Lincoln Bank & Trust Co., Louisville, Kentucky			$4,594.02
Deposit in Transit			824.49
Balance per Books—December 31, 1955			$5,418.51

B. BUDGETS FOR 1956-57. OFFICES OF SECRETARY-TREASURER AND ASSISTANT SECRETARY-TREASURER.

Direct Expenses:

Clerical	$ 250.00
Postage	150.00
Supplies	150.00
Printing	600.00
Travel and misc.	50.00
Novice award	100.00
Contingency fund	200.00
New membership list (production and distribution)	200.00
Gratuity	500.00
	$2,200.00

Note: This budget does not include the cost of printing abstracts and their handling. Because such has become customary in past years, it does include cost of printing programs and their distribution. The amounts in each of the categories of direct expense and for the new membership lists are approximate. Transfers from one category to another as necessary are approved.

Publication Committee Budget for 1956-57*

Extra pages in the JOURNAL	$1,000
Editor's office	1,000
Business manager's office	500
	$2,500

C. OFFICERS ELECTED FOR 1956-57 YEAR.

President	Joseph F. Volker
President-elect	Reidar F. Sognnaes
Vice-president	Ned B. Williams
Secretary-Treasurer	Edward H. Hatton
Asst. Secretary-Treasurer	Dan Y. Burrill
Trustee	George C. Paffenbarger
Editor	Hamilton B. G. Robinson
Publication Committee	Paul E. Boyle
	J. Frank Hall

Number of members voting—297.

D. NEW MEMBERS ELECTED 1956.

NORTH AMERICA—JOHN T. ALBRIGHT, Boston, Mass.; LESTER D. APPERSON, New Jersey; AINA M. AUSKAPS, Boston, Mass.; G. C. BATTISTONE, Washington, D. C., LISBETH G. BAUMANN, Philadelphia, Pa.; WILLIAM BIEDERMAN, New York, N. Y.; JACK G. BISHOP, Dallas, Tex.; RITA BOGOROCH, Boston, Mass.; F. J. BRAUER, Bethesda, Md.; MILTON L. BRAUN, Chicago, Ill.; H. K. BROWN, Ottawa, Canada; CLAIRE L. BURNS, Washington, D. C.; FRANCIS R. BURNS, Washington, D. C.; J. A. CAMERON, Dallas, Tex.; LLOYD E. CHURCH, Bethesda, Md.; PATRICIA G. CLARK, Indianapolis, Ind.; D. WALTER COHEN, Philadelphia, Pa.; HENRY I. COPELAND, JR., Washington, D. C.; HAROLD R. ENGLANDER, Great Lakes, Ill.; JOHN E. FOLK, Bethesda, Md.; JACOB B. FRANKLIN, Milwaukee, Wis.; ROBERT J. GORES, Rochester, Minn.; TOM J. GUPTA, Boston, Mass.; B. FRANKLIN GURNEY, Chicago, Ill.; SOL HABERMAN, Dallas, Tex.; VICTOR HALPERIN, New Orleans, La.; LOUIS S. HANSEN, San Francisco, Cal.; STANLEY C. HARRIS, Chicago, Ill.; JOHN I. INGLE, Seattle, Wash.; JOHN F. JOHNSTON, Indianapolis, Ind.; WILLIAM J. KING, River Edge, N. J.; ARTHUR KIRSCHBAUM, Houston, Tex.; MARGUERITE F. LITTLE, Rochester,

*This budget is based on the anticipated receipts from Supporting Associates. Any additional revenue will be used for additional pages.

N. Y.; DONALD L. MC ELROY, Chicago, Ill.; E. CARL MILLER, E. Cleveland, O.; CLIVE I. MOHAMMED, Chicago, Ill.; ALVIN L. MORRIS, Rochester, N. Y.; ARNE G. NIELSEN, Bethesda, Md.; J. V. NIIRANEN, Bethesda, Md.; W. A. NOLTE, Houston, Tex.; ALFRED L. OGILVIE, Seattle, Wash.; FLOYD D. OSTRANDER, Ann Arbor, Mich.; K. J. PAYNTER, Toronto, Canada; SAMUEL C. PECKHAM, Bethesda, Md.; DONALD R. PORTER, Portland, Ore.; ALBERT G. RICHARDS, Ann Arbor, Mich.; RICHARD A. RIEDEL, Seattle, Wash.; SEYMOUR W. SACHS, Washington, D. C.; RICHARD J. SCHNELL, Indianapolis, Ind.; ABRAHAM SCHWARTZ, Providence, R. I.; MAX SHAPIRO, Beverly Hills, Cal.; IRVING M. SHEPPARD, New York; MERRILL J. SHEPRO, Washington, D. C.; TETSUO SHIOTA, Bethesda, Md.; NORMAN THOMAS SPECK, Houston, Tex.; WILLIAM R. STANMEYER, New London, Conn.; RALPH R. STEINMAN, Loma Linda, Cal.; PAUL E. TULLAR, Houston, Tex.; GEORGE M. YAMANE, Washington, D. C.

OUTSIDE NORTH AMERICA—E. B. BRAIN, England; H. S. MALCOLM CRABB, England; A. D. DIXON, England; HARDY EBLING, Brazil; F. E. LAWTON, England; J. M. MUMFORD, England; S. L. ROWLES, England; MARIE R. WASDELL, England. Total number elected—67.

E. NECROLOGY

J. T. Gore
Robert Keilty
William F. Lasby
Howard Marjerison
E. G. Miller, Jr.
Theodore H. Rider
H. J. Turkheim (London)

F. STATISTICS OF 1956 MEETING

Members attending	236
Newly elected members attending	31
Non-members attending	274
Guests	43
Total attendance	584

The next general meeting of the Association will be held on March 21, 22, 23, 24, 1957, at the Claridge Hotel, Atlantic City, N. J. Abstracts of papers for inclusion on the program must reach the Assistant Secretary-Treasurer, Dan Y. Burrill, 129 E. Broadway, Louisville 2, Ky., not later than Dec. 1, 1956. The abstracts must be in triplicate, double-spaced, and not over 250 words. Items will be considered for the program only if they are received in proper form and ready for final publication and before the deadline.

Scientific exhibits will be part of the program at the 1957 meeting.

SUPPORTING ASSOCIATES*

OF

THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

AND

THE JOURNAL OF DENTAL RESEARCH

Acralite Company, Inc., New York, N. Y.
Julius Aderer, Inc., New York, N. Y.
Baker & Company, Inc., Newark, N. J.
Bristol-Myers, New York, N. Y.
L. D. Caulk Company, Milford, Del.
Coe Laboratories, Inc., Chicago, Ill.
Colgate Palmolive Company, Jersey City, N. J.
Cook-Waite Laboratories, Inc., New York, N. Y.
Crescent Dental Manufacturing Company, Chicago, Ill.
Densco, Incorporated, Denver, Colo.
Dental Materials Group, I.A.D.R., Indianapolis, Ind.
Dentists' Supply Company of N. Y., York, Pa.
Hanau Engineering Company, Inc., Buffalo, N. Y.
International Nickel Company, New York, N. Y.
J. F. Jelenko & Company, Inc., New York, N. Y.
H. D. Justi and Sons, Inc., Philadelphia, Pa.
Luxene, Inc., New York, N. Y.
The J. M. Ney Company, Hartford, Conn.
Precious Metals Research Works, Inc., New York, N. Y.
Procter & Gamble Company, Cincinnati, Ohio
Ritter Company, Inc., Rochester, N. Y.
Ticonium Division, Consolidated Metal Products Corp., Albany, N. Y.
The S. S. White Dental Manufacturing Company, Philadelphia, Pa.
Williams Gold Refining Company, Inc., Buffalo, N. Y.

*Supporting Associates have made contributions to the Association for support of the JOURNAL. These contributions are in multiples of one hundred dollars.